ECOLOGY AND EVOLUTION OF MUTUALISTIC NETWORKS

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SAMENVATTING

Mutualistische interacties komen overal in de natuur voor. Mutualistische interacties zorgen voor positieve terugkoppelingen tussen soorten, die weer van invloed zijn op de omgeving en evolutie van ecologische gemeenschappen. Ondanks de lange historie van ecologische en evolutionaire studies over mutualisme, wordt pas sinds kort erkend dat mutualistische interacties ook belangrijk zijn voor de modulatie van het functioneren van ecosystemen en voor het behoud van biodiversiteit. Mutualistische gemeenschappen, zoals bijvoorbeeld plant-bestuiver gemeenschappen, kunnen worden beschreven als een netwerk van interacties tussen mutualistische partners (bijv. planten en bestuivers). Met behulp van deze aanpak hebben vele studies hun licht kunnen schijnen op de structuur van mutualistische gemeenschappen en hun eco-evolutionaire patronen. Een aantal vragen blijft echter nog onbeantwoord: Welke ecologische en evolutionaire processen en mechanismen bepalen de samenstelling van mutualistische gemeenschappen? En welke van deze processen is het meest belangrijk voor de stabiliteit en diversiteit van deze gemeenschappen? Deze vragen worden in deze thesis behandeld. Het doel van deze thesis is een licht te schijnen op de dynamica en structuur van mutualistische netwerken.

Om deze vragen te kunnen beantwoorden hebben we wiskundige modellen en simulaties ontwikkeld en deze gecombineerd met data analyse. We hebben dit gedaan voor een scala aan mutualistische systemen, waarbij we vooral gefocust hebben op mutualistische plant-dier interacties. We hebben gevonden dat spatio-temporele variabiliteit de belangrijkste factor is van de mutualistische netwerk structuur. De combinatie van ruimtelijke structuur, abundantieverdeling en de variabiliteit in de timing van interacties tussen soorten (fenologie) is wat het meest de mutualistische netwerkstructuur bepaald. Mutualistische netwerken worden dus gevormd door zowel niche- als neutrale processen. De stabiliteit en diversiteit van deze gemeenschappen hangt echter af van de balans van deze processen met andere typen ecologische interacties (bijv. competitie) en de life-history van de soort (bijv. de populatiestructuur). Bovendien blijkt het dat veel voorkomende evolutionaire patronen in mutualistiche netwerken, zoals evolutionaire convergentie en complementariteit, lijken te ontstaan uit simpele ecologische (bijv. dispersielimitatie en ecologische drift) en evolutionaire (bijv. mutatie, recombinatie en genetische drift) processen. We concluderen dat: 1) neutrale eco-evolutionare processen niet moeten worden genegeerd bij het bestuderen van de evolutie van ecologische netwerken en 2) ruimtelijke processen (dispersielimitatie, immigratie) en fenologie van essentieel belang zijn voor de samenstelling van mutualistische netwerken, en waarschijnlijk voor ecologische netwerken in het algemeen. Veranderingen in het landschap ten gevolge van habitatdestructie en de verstoring van soort-interacties ten gevolge van veranderingen in het klimaat kunnen daarom de topologie van mutualistische netwerken dramatisch beschadigen. Dit zal uiteindelijk belangrijke ecologische functies (bestuiving, zaadverspreiding) voor ecosystemen aantasten.

SUMMARY

Mutualistic interactions are ubiquitous in nature. They create positive feedbacks between species, which can modify the environment (i.e. ecosystem engineering) and the evolution of ecological communities. Nevertheless, despite the long history of studies on mutualism in ecology and evolution, it was just recently recognized to be an important for network connectance and modulation of ecosystem function as well as for the maintenance of biodiversity. Mutualistic communities, such as plant-pollinator communities, can be described as a network of interactions between mutualistic partners (e.g. plants and animals). Using this network approach various studies have uncovered the structure of mutualistic communities and their eco-evolutionary patterns. Open questions are still: What ecological and evolutionary processes and mechanisms underlie the assembly of mutualistic communities? And which of those processes are more important for the stability and diversity of these communities? These questions are addressed in this thesis, which intends to create a general understanding of the dynamics and structure of mutualistic interaction webs.

We developed mathematical and simulations models and combined them with data analysis to explore these questions in a range of mutualistic systems, mainly focusing on plant-animal mutualistic interactions. We found that spatio-temporal variability is the main driver of mutualistic network structure. It is therefore the combination of spatial structure, species abundance distribution and the variation in the timing of species interactions (i.e. phenology) that seems to predominantly affect mutualistic network structure. Thus, mutualistic webs are shaped by both niche and neutral processes. However, the stability and diversity of these communities highly depend on the balance with other types of ecological interactions (e.g. competition) and the life-history of the species (e.g. population structure). Furthermore, common evolutionary patterns in mutualistic webs, such as evolutionary convergence and complementarity, seem to emerge from simple ecological (e.g. dispersal limitation, ecological drift) and evolutionary (e.g. mutation, recombination, genetic drift) processes. We conclude that: 1) neutral eco-evolutionary processes should not be disregarded when studying the evolution of ecological networks and 2) spatial processes (e.g. dispersal limitation, immigration) and phenology are essential for the assembly of mutualistic networks, and probably for ecological networks in general. Therefore, changes in the landscape by habitat destruction and the disruption of species interactions produced by climate change can dramatically damage the topology of mutualistic networks, which will ultimately impair important ecological services (e..g pollination, seed dispersal) for ecosystem functioning.

RESUMEN

Las interacciones mutualistas están presentes en todos los rincones de la naturaleza. Ellas crean retroalimentaciones positivas entre las especies que pueden modificar el ambiente (i.e. ingeniería de ecosistemas) y la evolución de las comunidades ecológicas. Sin embargo, a pesar de la larga historia de estudios sobre la ecológica y la evolución de los mutualismos, tan solo fue recientemente reconocida su importancia para la conectancia y modulación en el funcionamiento de los ecosistemas, así como para el mantenimiento de la biodiversidad. Las comunidades mutualistas, como por ejemplo las comunidades de plantas y polinizadores, pueden ser descritas como una red de interacciones entre especies mutualistas (i.e. especies que interaccionan beneficiándose mutuamente). El uso de este enfoque basado en redes ha ayudado a desvelar la estructura de las comunidades y sus patrones eco-evolutivos. Preguntas aun abiertas son: ¿Cuáles son los procesos y mecanismos detrás del ensamblaje de las comunidades mutualistas y cuáles de esos procesos son mas importantes para la estabilidad y diversidad de estas comunidades? Estas son las preguntas que hemos abordado en esta tesis, la cual pretende crear un entendimiento general de la dinámica y estructura de las redes mutualistas.

Nosotros desarrollamos modelos matemáticos y de simulación combinándolos con análisis de datos para explorar estas preguntas en diferentes sistemas mutualistas, principalmente focalizado en interacciones planta-animal. Los resultados muestran que la variabilidad espacio-temporal es el factor principal de la estructura de las redes mutualistas. Por lo tanto, es la combinación de estructura espacial, abundancia de las especies y la variación en el tiempo de las interacciones entre especies (i.e. fenología) la que parece afectar predominantemente la estructura de la red. Esto nos lleva a concluir que estas redes son moldeadas por procesos neutrales y de nicho. Sin embargo, la estabilidad y diversidad de estas comunidades depende altamente del balance con otras interacciones ecológicas (por ejemplo, competencia) y la historia de vida de la especies. Además, encontramos que los patrones evolutivos observados en redes mutualistas, como convergencia y complementariedad evolutiva, parecen emerger de procesos 'simples' ecológicos (e.g. deriva ecológica, dispersión limitada) y evolutivos (e.g. emutación, recombinación, deriva génica). Nosotros concluimos que: 1) procesos neutrales eco-evolutivos no pueden ser ignorados cuando se estudia la evolución de las redes ecológicas y 2) que los procesos espaciales (dispersión limitada, inmigración) y la fenología son factores esenciales para el ensamblaje de las redes mutualistas, y probablemente para las redes ecológicas en general. Por lo tanto, cambios en el paisaje por destrucción de hábitat y la interrupción de las interacciones entre especies por el cambio climático actual pueden dañar dramáticamente la topología de estas redes, lo cual finalmente afectara negativamente importantes servicios ecológicos (e.g. polinización, dispersión de semillas) para el funcionamiento del ecosistema.

1

INTRODUCTION

> "*It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us.*" Darwin (1862b)

1.1 MUTUALISM

Mutualistic interactions are ubiquitous and crucial for the functioning of ecosystems and the maintenance of biodiversity. They are an essential part of the entangled bank that Darwin mentioned in his masterpiece: *The origin of the species* (Darwin, 1862b). They provide important ecosystems services and enable the survival of many angiosperm plant species. Mutualism seems to be responsible for the amazing diversification of angiosperm plants (Barrett, 2008) and primates (Gómez and Verdú, 2012). Positive feedbacks in nature, such as mutualistic interactions, occur more frequently than we think, particularly through indirect effects (i.e. indirect mutualisms), for example in plant-herbivore interactions, which can be an indirect mutualism through the presence of soil decomposers that recycle nutrients (Loreau, 2010; de Mazancourt et al., 1999). Recently, a three-stage symbiosis has been reported that is crucial for seagrass ecosystems. The symbiosis involves seagrass, lucinid bivalves and their sulfide-oxidizing gill bacteria, which reduce sulfide stress for seagrasses and improve seagrass production (van der Heide et al., 2012). Nevertheless, other ecological interactions, such as competition and trophic interactions (e.g. predator-prey) have historically been studied more in the ecological literature on factors structuring communities (Pimm, 1979; Tilman, 1982; Hairston et al., 1960). The importance of mutualism and other non-trophic interactions (e.g. facilitation) has only been relatively recently acknowledged as crucial for the maintenance of biodiversity and ecosystem functioning (Bertness and Callaway, 1994; Stachowicz, 2001).

Mutualism is defined as a cooperation between species by the exchange of goods and/or services (Bronstein et al., 2006). Goods can be nutrients or resources, such as pollen and fruits, and ecological services provided by mutualists can be pollen/seed dispersal or protection against herbivores. Mutualistic interactions can be divided into three main types according to what benefits are exchanged: 1) resource-resource mutualisms (plant-mycorrhizal, plant-rhizobium), 2) resource-service mutualisms (plant-pollinator) and 3) service-service mutualisms (protection-protection, cleaning-protection). In this thesis I will mainly focus on resource-service mutualisms. This type of mutualism is very common and it includes ecologically important interactions such as plant-pollinator and plant-animal seed-disperser interactions. I will also dedicate one chapter to plant-mycorrhizal interactions, which are based on exchange of resources. This type of mutualism tends to be more labile, varying from antagonistic to mutualistic, and seems to mainly depend on the local abiotic conditions (Johnson et al., 1997a).

1.2 PLANT-ANIMAL MUTUALISMS

Classical studies of plant-animal mutualisms are based on highly specialized mutualism (i.e. obligatory mutualisms), such as the fig-fig wasp (Cook and Rasplus, 2003) or the yucca-yucca moth (Pellmyr, 2003) system. The study of these specialized interactions has provided empirical and theoretical understanding of the ecological stability and coevolution of mutualistic interactions (Bronstein et al., 2003b; Holland, 2002). Classical theoretical studies on mutualism concluded that mutualism tend to be inherently unstable (May, 1976). Positive feedbacks make species grow to infinity, but of course this has some limits (Ulanowicz and Hannon, 1987). Naturally, the benefits of mutualism are not infinite, on the contrary they are constrained by different costs associated with the interaction (Holland, 2002; Addicott, 1981). Subsequent studies showed that if we impose that the benefits of mutualism must be finite, the interaction becomes stable (Dean, 1983; Addicott, 1981; Soberon and Martínez del Río, 1981). Thus, it is the balance between costs and benefits that stabilizes the interaction. For example, mutualism has been intimately related to the idea of trading in economics as Noe and Hammerstein (1994) explained, where the balance between benefits and costs mediates the stability of the interaction. Darwin (1862a) was the first to thoroughly study the intriguing cooperation between plant and animal species. He wondered whether such interactions could be explained in terms of selfing actions and he realized that conflicts of interest between partners were essential factors for mutualism to work (Bronstein et al., 2006). More recently, there is an increasing interest to develop a more robust and general theory of mutualistic interactions (Holland and DeAngelis, 2010; Holland et al., 2002).

Plant-animal mutualisms, in general, can be formulated in terms of consumer-resource interactions (Holland and DeAngelis, 2010; Chamberlain and Holland., 2008), where consumers (e.g. pollinators) get resources from the plants (e.g. nectar) and the plants receive in exchange benefits through reproduction (e.g. pollen or seed dispersal). An important feature of plant-animal mutualisms, which have been usually ignored from most theoretical studies is the life-history of the species. For example, most pollinators are insects with complex life cycles and the consideration of pollinator life-stages can probably influence the stability of plant-pollinator interactions.

Furthermore, most common plant-animal mutualistic communities are composed of multiple interacting species and with different levels of specialization (Jordano, 1987; Vázquez and Aizen, 2004) and most species in plant-animal mutualistic communities are facultative mutualists (Jordano et al., 2003) (i.e. mutualist species that do not strictly depend on the presence of a specific partner species). Thus, contrary to the rare case of obligatory mutualisms, such as the fig-fig wasp interactions; most plant and animal mutualists interact with several partner species. The western honey bee (*Apis mellifera*) is a good example of facultative mutualism with high level of generalization and they are crucial for many species crop-pollinated plants (Kremen et al., 2002).

Plant-animal mutualistic communities are well studied empirically and their structure can be described as a network of species interactions, where nodes represent species and the links connecting nodes represent observed interactions between plants and animals (Bascompte and Jordano, 2007a). Therefore, these networks are composed of two sets of species or guilds: plants and animals, where there are only mutualistic interactions between guilds. Networks

composed of two set of nodes are defined in graph theory as bipartite graphs. Mutualistic networks are a good example of bipartite graphs (or networks), similar to some economic networks, such as bank-company networks (Souma et al., 2003). Empirical and theoretical research on mutualistic networks have unveiled the importance of considering the intricate structure of interactions between mutualistic partners for the maintenance of biodiversity and ecosystem functioning.

1.3 MUTUALISTIC NETWORKS

Graph theory and complex network theory have helped to describe and understand complex systems[1]. Different scientific fields, such as physics, economics, sociology and biology have benefited from the mathematical development in graph theory and their applications to different systems (e.g. ecosystems, stock-markets) by complex network theory (Newman, 2003). Biological organisms are complex systems, which interact with other agents (i.e. individuals) and are composed of fairly complex metabolic pathways. Part of this complexity can be described as a network, which is a set of nodes interconnected among them by links. Nodes can be genes or individuals and links can described as interactions between nodes. Thus, these theories have helped us to describe different biological networks, such as gene-regulatory networks (Levine and Davidson, 2005), protein-protein networks (Maslov and Sneppen, 2002) and ecological networks (Solé and Montoya, 2001).

Ecological networks describe interactions between species and/or across space (i.e. spatial networks (Urban and Keitt, 2001)). We can classify ecological networks according to their type of ecological interaction, for example, host-parasite networks, trophic networks (i.e. food webs)(Pimm, 1979) or mutualistic networks (Jordano, 1987). Studies on mutualistic networks are more recent than studies on trophic networks (i.e. food webs) and ecosystems (Bascompte and Jordano, 2007b). Traditionally, ecosystems and communities have been described as networks of energy fluxes by Odum (1968) andMargalef (1963). Food webs also have a long tradition in ecology (Cohen, 1978; Pimm, 1979) and have prevailed in ecological research as a way of describing community structure (Dunne et al., 2002). However, in the last decade, mutualistic networks have produced great progress in community ecology highlighting the importance of mutualism for the maintenance of biodiversity (Bascompte et al., 2006; Bascompte and Jordano, 2007b). Most studies on mutualistic networks have focused on plant-animal mutualisms (e.g. plant-pollinator)(Bosch et al., 2009), although more recently the network of interactions in other mutualistic systems, such as plant-mycorrhizal interactions (Montesinos-Navarro et al., 2012), have started to receive attention.

1.3.1 *Topological properties*

One important feature of complex systems is the presence of emergent properties. Emergent properties are the result of the interactions among the components or parts of the system; therefore these properties are a result of the global behavior of the system (Sole and Bascompte, 2006). Many of the topological properties found in mutualistic networks are

1 Complex systems is a system composed of interconnected parts that as a whole exhibit one or more properties (behavior among the possible properties) not obvious from the properties of the individual parts (Wikipedia)

emergent properties that result from the complex interaction between plants and animals across time and space (Vázquez et al., 2009a). Mutualistic networks have several unique topological properties that differentiate it from other ecological networks. I will now explain each of these unique properties:

1.3.1.1 *Degree distribution*

One important feature of complex networks is that their distribution of number of links per node, i.e. its degree distribution, is characterized by having many nodes with few links and very few nodes with many links (Sole and Montoya, 2001). This means that complex networks have very heterogeneous degree distributions and this degree distribution can be mathematically described by a power-law function: $p(k) \propto k^{-\gamma}$, where $p(k)$ is the probability of a node of having k links and γ is the critical exponent. This type of distribution is called scale-free because the relationship between k and $p(k)$ is not defined by a particular scale (Barabasi and Albert, 1999).

Mutualistic networks have a degree distribution better described by a truncated power-law function:

$$p(k) \approx k^{-\gamma} e^{-k/k_c}$$

where k_c is the critical connectivity and the exponential term is the cutoff, when k approaches to k_c, $p(k)$ decreases faster than the power-law function (Bascompte and Jordano, 2007b). This means that mutualistic networks are still much more heterogeneous than expected by chance. Many species have with few interactions and a few species have a very large number of interactions. However, they are not as heterogeneous as scale-free networks and this is probably related to different constraints (biological or not) affecting network topology. This degree distribution makes mutualistic networks more robust to loss of key stone species (Sole and Bascompte, 2006).

1.3.1.2 *Nestedness*

The concept of nestedness originated in the island biogeography literature of Atmar and Patterson (1993) where it was used to describe the distribution of species across islands. In the mutualistic network literature, nestedness describes a non-random pattern of species interactions where specialist species interact with proper subsets of more generalist species (Bascompte et al., 2003). This is illustrated in Figure B.6. Bascompte and Jordano (2007b) describe two important features of nested networks: 1) there is a core of generalist species interacting among them and 2) specialists tend to interact with the most generalist species, so there is high asymmetry in terms of specialization levels. Mutualistic networks in general are highly nested (Jordano et al., 2003; Dupont and Olesen, 2009; Bascompte et al., 2003; Montesinos-Navarro et al., 2012) and several studies suggest that nestedness increases the stability and coexistence of mutualistic communities (Bastolla et al., 2009; Okuyama and Holland, 2008). However, another study found the opposite: nested networks tend to promote instability compared to unstructured networks because of their resulting interaction strength distribution (Allesina and Tang, 2012). Furthermore, nestedness seems to be the

product of multispecific coevolutionary processes (Bascompte et al., 2003), although neutral processes can also produce nested structures (Krishna et al., 2008).

1.3.1.3 *Modularity*

Networks can have regions of nodes that are more densely connected than others. These regions are called *modules* or *compartments*, while less connected regions set the boundaries of the modules (Newman, 2006). Modularity is essential in biological systems. Organisms are generally organized into modules where different subsets of units have a specific functionality. An example is provided by modules of genes involved in development (Gu, 2009). Modularity reveals the underlying structure in the network, which is relevant to detect groups of significant importance (Newman, 2006). In ecological networks we can find modules of species that are highly interacting among them but weakly between modules (Pimm and Lawton, 1980). Modularity describes the extent to which species are organized into modules (Newman, 2006). In mutualistic networks (and other ecological networks), modularity can emerge from spatio-temporal structure and/or evolutionary processes leading to non-random patterns of interactions (Olesen et al., 2007). Modules have been suggested to be units of coevolution and some phylogenetic studies support this idea (Rezende et al., 2007b). Plant-animal mutualistic networks are modular (Olesen et al., 2007). Generalist species connect peripheral species together into modules, but also connect modules keeping the cohesiveness of the network. These generalist species act as modular hubs and they are crucial to maintain the cohesiveness of the network (Guimaraes et al., 2007). The extinction of a module hub can lead to fragmentation of the network. Therefore, they are considered very important for the conservation of mutualistic communities. Modular networks also tend to be less affected by disturbance because disturbance spread slower in a highly modular network(Olesen et al., 2006). Interestingly, invader species, usually generalists, may cause fusion of modules with profound effects on network functioning and structure.

1.3.1.4 *Asymmetry*

The network properties mentioned above share a common feature: they are often asymmetrical. Asymmetry is pervasive at all levels in mutualistic networks, from species interactions to interaction strengths (Vázquez and Aizen, 2004). Nestedness shows highly asymmetrical patterns of interactions; i.e. specialist interact more frequently with generalist partner species and the degree distribution is heterogeneous (i.e. high proportion of species with few interactions and low proportion of species with many interactions). Moreover, the distribution of interaction strengths is also highly asymmetrical between plants and animals (Bascompte et al., 2006). Interaction strength (or species dependence) in plant-animal mutualisms is usually measured by the relative frequency of interactions (Bascompte and Jordano, 2007a). For example, in plant-pollinator communities it is estimated by the relative frequency of floral visits (Jordano, 1987). Similarly, the dependence of an animal species on a plant species can be estimated as the relative frequency of fruits consumed from that particular plant species. The frequency distribution of species dependence is highly positively skewed, i.e. there are many weak dependences and few strong ones (Bascompte et al., 2006; Jordano,

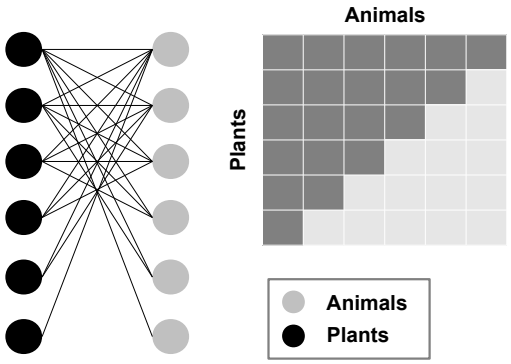

Figure 1.1: Nested networks. A perfectly nested structure of a mutualistic web is represented by a plant-animal interaction matrix (right side) and a network cartoon (left side). The interaction matrix shows plant species in rows and animal species in columns, where dark gray squares represent observed interactions between a plant and an animal species and light gray squares are non-observed interactions. This matrix is perfectly nested because specialist species form perfect subsets of more generalized species interacting with their mutualistic partners. The right side of the figure shows an explicit representation of the network, where black nodes represent plant species and gray nodes represent animals species and the lines connecting these nodes represent the observed interactions.

1987). This pattern has also been reported in food webs (Bascompte et al., 2005; Paine, 1980; Wootton, 1997). Theoretical studies suggest that this distribution of interaction strengths promotes community persistence and stability in food-webs (Bascompte et al., 2005; May, 1973; Berlow et al., 2004). Furthermore, the dependences between plants and animals are highly asymmetric (Vázquez and Aizen, 2004; Bascompte et al., 2006). This means that, for example, some plant species are highly dependent on the service (e.g. pollination) provided by an animal species, but this animal species might depend much less on the resources (e.g. nectar) provided by the plant (Bascompte et al., 2006).

1.3.2 *Mechanisms and processes for the assembly of mutualistic webs*

We have seen that mutualistic network structure is not random and has very specific properties. Therefore, questions arise such as: What are the assembly rules behind the structure of plant-animal mutualistic communities? What ecological and/or evolutionary processes and mechanisms are shaping these webs? Are there some processes more important than others?

These are the fundamental research questions of this thesis. Several studies have tried to answer these questions combining empirical and theoretical research. Most of the evidence suggest that niche-driven processes are mainly responsible of the observed structure (Jordano et al., 2003; Bascompte and Jordano, 2007b; Santamaría and Rodríguez-Gironés, 2007; Stang

et al., 2006). However, other evidence indicate that neutral processes have an important influence on those observed patterns (Krishna et al., 2008; Canard et al., 2012; Vázquez et al., 2007).

1.3.2.1 *Niche processes and forbidden links*

The traditional view is that specialization, through selection regimes, to local environmental conditions is what shapes communities and determines their diversity and composition (Futuyma, 1998; Hutchinson, 1973). Since Darwin's studies on orchids (**?**) the natural history of plant and animal mutualisms have shown us the importance of biological constraints for species interactions. Biological constraints, also called *forbidden links*, are then all those biological traits that do no allow interactions between species (Bascompte and Jordano, 2007b). The most important biological constraints are morphological and phenological constraints (Olesen et al., 2010; Stang et al., 2009; Vázquez et al., 2009c). Morphological constraints (or trait mismatches) refer to all those physical constraints due to mismatches between morphology of the species involved in the interaction (including body size)(Olesen et al., 2010; Stang et al., 2009). The classic example is the size differences between the corolla of a flower and the proboscis in Lepidopteran pollinators. To interact the proboscis should at least reach the bottom of the corolla to get the energetic reward (i.e. nectar). Another common example is the beak size of frugivorous birds and the size of the fruits (Wheelwright, 1985). Body size is also a well known physical constraint for the interaction between plants and animals and is allometrically related to other morphological traits (e.g. proboscis) (Kunte, 2007). In plant-pollinator networks, around 3% of all potential links can be forbidden because of morphological constraints; however in plant-seed disperser networks around 15% of all potential links can be due to morphological constraints (Olesen et al., 2010). Stang et al. (2007) found that morphological constraints were important factors shaping plant-pollinator webs. However, other factors, such as species abundance were also important (Stang et al., 2007; Vázquez et al., 2007).

Among all biological constraints, phenological uncoupling is undeniably one of the most important in mutualistic networks (Olesen et al., 2008, 2010; Vázquez et al., 2009a); and ecological networks in general (Post et al., 2008a; Post and Inouye, 2008). Phenology is a life-history trait that is especially important in organisms with complex life cycles and defines the period or time-frame of life-cycle events associated to seasonal environments (Stenseth and Mysterud, 2002; Yang and Rudolf, 2010). Common examples of phenological traits are flowering and fruiting time, the flying periods of migratory species or the period of activity of adult pollinators. Phenological uncoupling or mismatch occurs when species do not coincide or overlap temporally (Stenseth and Mysterud, 2002). For example, a plant species only flowering early in the season will never interact with a pollinator that is only active at the end of the season. Phenological uncoupling is the most important biological constraint representing 22 to 28 % of all non-observed interactions in plant-animal mutualisms and it is clearly more important in high latitude environments with strong seasonality (Olesen et al., 2010). Vázquez et al. (2009c) have shown that phenological uncoupling is the most important biological constraint shaping, and spatial structure is the second most important.

The temporal dynamics of plant-pollinator networks show that there is a high turn-over of species between years, but the species are substituted by functionally equivalent species

in terms of degree of specialization (Olesen et al., 2008; Petanidou et al., 2008). Therefore, network topology is not affected or modified by between-years species turn-over; main topological properties, such as nestedness and connectance, are maintained from year to year. Climate change can alter the phenology of the species, hence disrupting species interactions and range of distributions (Post et al., 2008a; Hegland et al., 2009; Inouye, 2008). These effects ultimately alter community structure and cascading effects, such as secondary extinctions (Nakazawa and Doi, 2012). Although various evidence point to the huge importance of phenology in the assembly of mutualistic communities, especially for their conservation due to the dramatic effects of climate change, we still do not know how phenology affects network structure and community dynamics.

Santamaría and Rodríguez-Gironés (2007) explored different null models to understand whether niche (i.e. forbidden links) or neutral processes were more important for the topology of mutualistic webs. They found that niche-driven mechanisms (i.e. biological constraints) were more important explaining network topology than in neutrally assembled communities. However, other studies have shown results supporting the importance of neutral processes (Krishna et al., 2008; Canard et al., 2012).

1.3.2.2 *Neutral processes*

The neutral theory of biodiversity postulates that dispersal, speciation and demographic stochasticity (i.e. ecological drift) are the main processes in the assembly of ecological communities (Hubbell, 2006, 2001; Alonso et al., 2006; Rosindell et al., 2011). The main assumption of this theory is that "all individuals within a particular trophic level have the same chances of reproduction and death regardless of their species identity" (Rosindell et al., 2011). Although we know that this is not true, this theory is able to predict well observed species-abundance distributions (SAD) and species-area relationships (SAR) (Rosindell et al., 2011). Neutral processes also seem to predict the structure of ecological networks (Canard et al., 2012). Various empirical evidence shows that the species abundance distribution is the main driver of the pervasive asymmetry in mutualistic network properties, such as nestedness or degree-distribution (Vázquez, 2005; Vázquez and Aizen, 2004; Dupont et al., 2003). Vázquez et al. (2007) proposed the "asymmetry-abundance" hypothesis to give a more parsimonious explanation for the observed network structure. This hypothesis states that species abundance distributions and random interactions between individuals are determinants of mutualistic network properties. The 'neutrality' ('ecological equivalence') assumption in ecological networks is that interactions between individuals, regardless of species differences, are random; i.e. all individuals have the same probability of interaction. Vázquez et al. (2007) tested this hypothesis and concluded that species abundance greatly contributes to the asymmetry structure of mutualistic networks. However, the study also suggests the role of other factors.

Theoretical studies have demonstrated that neutral processes can indeed produce similar network properties as those observed in nature (Krishna et al., 2008; Canard et al., 2012). There is evidence that spatio-temporal variability shapes the topology of mutualistic networks (Vázquez et al., 2009a; Dupont et al., 2009). Thus, a combination of niche and neutral processes might drive the structure and dynamics of mutualistic communities (see Figure 1.2). It seems that once biological constraints are taking into account, the systems look

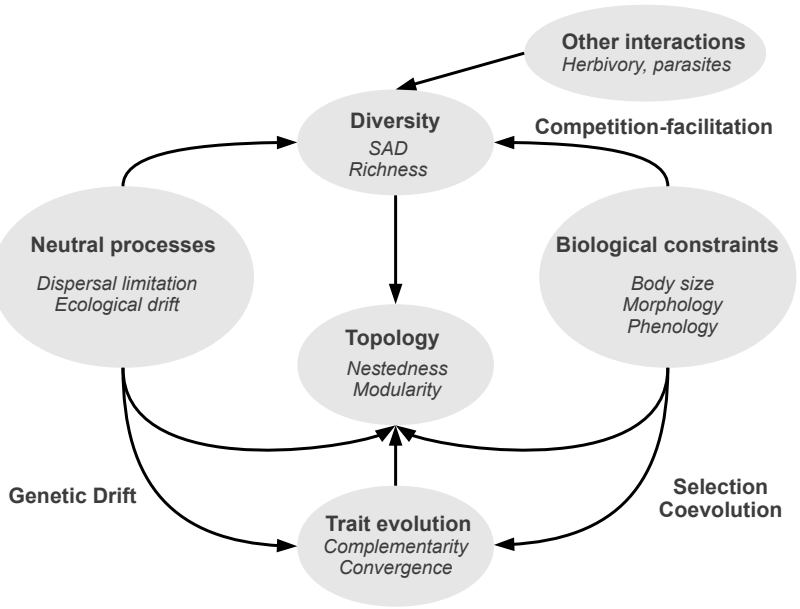

Figure 1.2: The assembly of mutualistic webs. The assembly of mutualistic webs follows a combination of niche and neutral eco-evolutionary processes. These processes act simultaneously in the metacommunity and hence they cannot be easily disentangled. On one hand, at the ecological level biological constraints and ecological interactions (trophic and non-trophic) jointly mold the diversity and topology of the network. On the other hand, neutral processes exert their influence on the diversity and topology of the network through processes such as ecological drift and dispersal limitation. At the evolutionary level, the joint action of selection and non-selective forces (e.g. genetic drift, recombination) produces changes in the individuals of the community. The feedbacks between ecology and evolution finally produce the emergent properties observed in real webs and these feedbacks change across space and time (i.e. they are dynamical).

neutrally assembled. However, it is still not clear what specific mechanisms are responsible of the assembly of mutualistic and how important neutral versus niche processes are. These open questions are addressed in this thesis.

1.3.3 *Network robustness*

The removal of keystone species could trigger secondary extinctions and cascading effects fragmenting the network into different subwebs (Montoya et al., 2006). Therefore, understanding the architecture of the network and identify those species is important to conserve and manage ecosystems (Solé and Montoya, 2001). Plant-animal mutualistic networks provide several ecosystem services that are highly important for plant reproduction. These services can be lost because of the extinction of important generalist species, such as the honey bee (Biesmeijer et al., 2006), and generate secondary extinctions in the community.

However, theoretical studies suggest that the structure of mutualistic webs is highly robust. Fortuna and Bascompte (2006) showed that mutualistic metacommunities with observed structures (i.e. highly nested) were more resistant to habitat loss than those with randomized network structure. Another study also showed that the structure of mutualistic webs is very robust against the extinction of species and concluded that both the truncated power-law degree distribution and the nested structure give high robustness to these webs (Memmott et al., 2004). The temporal mismatch in species phenologies due to climate change could fragment the network, potentially generating negative effects at the ecosystem level (Hegland et al., 2009). We still do not know how robust mutualistic webs are to the effects of climate change. Some studies report that communities in arctic communities are very fragile to the adverse effects of climate change (Post and Forchhammer, 2008).

1.3.4 *Evolution and coevolution of mutualistic webs*

Darwin was the first to think about the complex process of coevolution [2](Darwin, 1862b) and since then biologists have been trying to understand how species interactions generate trait changes. The first example of coevolution was studied by Darwin (**?**) showing that the long corolla of the orchid *Angraecum sesquispedale* could only be reached by a pollinator species (*Xanthopan morgani*) with a similar proboscis length. However, much later Janzen (1980) showed that this amazing example of coevolution and high specialization between plants and animals was not common. He explained that coevolution can also be the product of multispecific interactions, a term that he coined "diffuse coevolution". Diffuse coevolution means that selection on traits is generated by multiple interactions among species in the community (Strauss and Irwin, 2004). Therefore, the presence of a specific assemblage of animals with different morphological and sensorial traits can exert different selective pressures on plant traits (Florchinger et al., 2010). This is the idea of pollination and dispersal "syndromes", where plants have a set of traits that attract a specific group of animal species (e.g. pollinators or animal seed-dispersers) (Fenster et al., 2004; Howe and Smallwood, 1982a; Fischer and Chapman, 1993). The sensorial abilities of animals (pollinators or seed-dispersers) are the main drivers of the evolution of floral and fruit traits (Lomáscolo and Schaeffer, 2010; Florchinger et al., 2010). For example, empirical evidence suggests that different animal visual and olfactive senses are very important in the evolution of fruit diversification by frugivorous (Schaefer et al., 2007, 2008; Schaefer and Schmidt, 2004). However, to our knowledge, there are no theoretical studies showing the importance of these sensorial biases in the evolution of fruit or floral traits. In summary, various evidence supports the idea that diversification of plant-animal mutualistic systems is probably a product of multispecific interactions from diverse functional groups (e.g. primates, birds, bees, butterflies).

Several theoretical studies have analyzed the evolutionary and ecological conditions for the coevolution of plant-animal mutualistic systems (Ferdy et al., 2002; Ferriere et al., 2007; Law et al., 2001; Jones et al., 2009). These studies, usually based on specific pair-wise mutualistic systems (e.g. globeflower-flies, fig-fig wasp), have shown that the evolution of mutualism is greatly determined by intra- (Ferdy et al., 2002) and interspecific competition

2 defined as reciprocal evolutionary change between species

between mutualists and exploiters (Ferriere et al., 2007; Jones et al., 2009). Jones et al. (2009) showed that the coevolution of mutualistic systems can be highly stable if competition between mutualists and exploiters (i.e. cheaters, free-riders) is high. In contrast, when competition is weak, the system is driven to evolutionary suicide. This study emphasizes that coevolution in mutualistic systems needs the presence of antagonists to (co)evolve. However, in a multispecific context, we do not know what conditions are necessary to generate a high diversity of traits as for example observed in angiosperm plants (Herrera, 1989b). The evolution and coevolution of mutualisms highly depend on the local abiotic and biotic conditions in which the species pair occurs (Thompson, 1999; Thompson and Cunningham, 2002). In some mutualistic systems, such as plant-mycorrhizal interactions, the interactions are very labile and might fluctuate from mutualistic to antagonistic depending on local conditions (Sanders and Croll, 2010; Johnson et al., 1997b). Therefore, the coevolution of mutualistic systems may depend on the variation across space and time of fitness interactions among partner species (Gomulkiewicz et al., 2000).

The geographic mosaic theory of coevolution explains exactly this: coevolutionary dynamics change across space and time. This theory proposes that coevolution depends fundamentally on the spatial structure and dynamics of variable interaction effects (Thompson, 1999). The theory has two main components: selection mosaics and coevolutionary hot and cold spots (Thompson, 1999, 1997). Selection mosaics occur when natural selection varies among different communities. Hot spots are places where interacting species undergo strong selection with reciprocal effects on fitness and cold spots are places where selection affects only one species or none at all (Gomulkiewicz et al., 2000). There is empirical evidence showing the presence of coevolutionary hot spots in mutualistic systems (Benkman et al., 2001; Anderson and Johnson, 2007). The undeniable effect of spatio-temporal variability and multispecific interactions on the eco-evolutionary dynamics of mutualistic systems are the main factors to explain the observed structure and the evolutionary trait patterns in plant-animal mutualisms.

Different studies have suggested that the structure of mutualictic networks, mainly nestedness and modularity, can result from the evolution of phenotypic complementarity and convergence (Rezende et al., 2007a,b; Vázquez et al., 2009a; Santamaría and Rodríguez-Gironés, 2007). Complementarity refers to the evolution of similar traits (i.e. trait matching) between species; therefore, it is the product of coevolutionary processes (Nuismer et al., 1999). An example of complementarity would be the correlation between seed size and body mass of frugivores (Jordano, 1995) or between corolla and proboscis length(Muchhala and Thomson, 2009). Evolutionary convergence of traits is the evolution of similar traits due to common selective pressures. Thus, pollination and dispersal 'syndromes' reflect and predict convergent selection pressures on floral and fruit traits (Fenster et al., 2004; Howe and Smallwood, 1982a). Guimaraes et al. (2011) studied a model of coevolution of mutualistic networks and found that coevolutionary processes and common selective pressures can generate trait convergence and complementarity. However, their model did not consider changes in network structure by the evolutionary dynamics. Therefore, it is still not clear whether coevolutionary processes can produce similar network structure to the real ones. Furthermore, we may ask whether there are other evolutionary processes that could generate trait complementarity and convergence in mutualistic webs.

Neutral eco-evolutionary dynamics models have been able to partly explain the emergence of diversity and structure in metacommunities. These models consider explicitly genetics and space assuming basic principles of the neutral theory of biodiversity and neutral evolution, where natural selection is absent and all individuals are ecologically equivalent (Melián et al., 2012; de Aguiar et al., 2009; Melián et al., 2010). Therefore, these models assume neutral ecological processes (i.e. dispersal limitation, ecological drift and speciation) and non-selective evolutionary forces (i.e. genetic drift, mutation and recombination). It is clear that selection is a key evolutionary force in the evolution of organisms and can act at different levels, but the effect of non-selective evolutionary forces as drivers of evolutionary changes in populations is important as well . Again, it seems that multiple processes are driving the evolution of mutualistic networks; a combination of both selective and non-selective forces can influence the patterns observed (see Figure 1.2). The question is to what extent these neutral eco-evolutionary processes can explain current ecological and evolutionary patterns in mutualistic networks.

1.4 THESIS OVERVIEW

This thesis aims to study the ecological and evolutionary processes responsible for the diversity and structure of mutualistic networks. The focus of this thesis is mainly theoretical and mostly related to plant-animal mutualisms. My main goal is to shed light on the importance of niche and neutral processes behind the assembly of mutualistic communities and the importance of life-history in the ecology and evolution of plant-animal mutualisms. My approach uses population/community dynamics models and data analysis to gain insight in the importance of these processes.

In Chapter 2, we develop and analyze a simple model of plant-pollinator interactions that considers pollinator's life-history. This chapter aims to understand the importance of population structure for the stability of plant-pollinator interactions and the viability of pollinator populations suffering external mortality factors at different life-stages. Considering the life-history of specific mutualistic systems, such as plants and insect pollinators, can reveal important ecological conditions for management and conservation of pollination services. This is not only important for the functioning of natural ecosystems, but also for the economical role that mutualisms play in agriculture (crop-pollinated plants).

Chapter 3 studies the evolution of fruit traits that attract frugivores by considering the life-history of the plants and the foraging behavior of animals. We used a spatially explicit individual-based model to study the evolution of fruit traits. This model is the first, to our knowledge, to explore the conditions for the evolution of fruit traits to attract frugivores. It is, therefore, a step forward in understanding the evolution of plant-animal seed-disperser mutualisms and the emergence of 'dispersal syndromes'.

Chapters 4, **5** and **6** explore the importance of niche and neutral processes in the assembly of mutualistic communities.

Chapter 4 explores the importance of phenology as a mechanism for the assembly of mutualistic networks. Phenology is the most important biological constraint shaping the structure of mutualistic webs and probably also has an important role in the dynamics of the network. In this chapter, we develop and analyze a model that considers phenological

couplings (i.e. temporal overlap between two species) as a proxy for interspecific interaction strength to study the dynamics and structure of mutualistic networks. In the model we consider mutualistic and intraguild competitive interactions to model the community dynamics and explore the effect of different phenological distributions (i.e. configurations of multiple species phenologies spread across a season) on the dynamics. We also use realistic estimates of phenology distributions from empirical data of plant-pollinator communities.

Chapter 5 studies the main processes behind the topology of plant-mycorrhizal networks. In this chapter we use a spatially explicit data set of a plant-mycorrhizal community and apply different randomization tests (i.e. null models) to disentangle what ecological processes (e.g. dispersal limitation, habitat filtering) are important in structuring the observed network. We also develop and apply novel metrics of spatial overlap as a proxy for species interaction.

Chapter 6 explores the eco-evolutionary dynamics of mutualistic networks. This model aims to understand the importance of simple ecological and evolutionary processes shaping the topology of mutualistic webs and the emergence of evolutionary trait patterns (i.e. phenotypic convergence and complementarity). The model considers space and genetics explicitly, the expression of quantitative traits and morphological constraints (i.e. forbidden links) for the interactions between plants and animals.

Finally, **Chapter 7** is a synthesis of the main results and a discussion of the future theoretical and empirical perspectives in the mutualistic network literature.

2

POLLINATOR POPULATION STRUCTURE CREATES COLLAPSE OF POLLINATION SERVICE

Francisco Encinas-Viso, Tomás A. Revilla and Rampal S. Etienne

Most pollinators are insects with several life-stages (e.g. larva, adult) and their mutualistic interaction depends on surviving these life-stages. However, pollinator population structure has been poorly studied theoretically, and we lack understanding of the influence of different life-stages on the stability of the mutualism. Here we present a plant-pollinator model where the mutualism is facultative for the plant and obligatory for the stage-structured pollinator. Our model predicts a globally stable equilibrium when pollinator demography is dominated by adults and a locally stable equilibrium when the plants are strongly dependent on pollination and pollinator demography is dominated by the larval stage. In the latter case, the mutualism is vulnerable to fluctuations in population size or structure caused by external factors (e.g. pesticides) reducing larval development and increasing adult mortality. This may cause a sudden collapse of the mutualism, after which restoration of the pollination service not only requires reduction of these external factors but also large increases in pollinator populations.

Keywords: mutualism, pollination service, population structure, pesticides, Allee effect, bistability

Submitted

INTRODUCTION

Plant-pollinator interactions are essential for ecosystem functioning and the maintenance of biodiversity (Balvanera et al., 2005). Many angiosperm plants depend on the service provided by pollinators to reproduce (Kearns et al., 1998). Empirical studies of this type of mutualistic interaction are abundant (Waser, 2006). However, theoretical studies of plant-pollinator interactions are relatively scarce, originally focusing on very specific systems (e.g. fig-fig wasp) (Bronstein et al., 2003b; Wilson et al., 2003) but more recently on mutualistic community dynamics (Okuyama and Holland, 2008; Bastolla et al., 2009). Holland and DeAngelis (2010) have proposed to study plant-pollinator systems, and other types of mutualism (e.g. plant-mycorrhiza), in terms of consumer-resource interactions to develop more mechanistic models of mutualism. The theory of plant-pollinator interactions is progressing (Bronstein et al., 2006; Bascompte and Jordano, 2007b; Holland et al., 2004b, 2002), but a crucial component of this interaction is missing in many theoretical studies: the consideration of population structure. Many pollinators are insects with complex life-cycles, i.e. they have several life-stages (e.g. egg, larva, pupa, adult) and each life-stage is subject to different selective pressures (Wilbur and Rudolf, 2006; Herrera, 1984) and can have multiple indirect effects on their mutualistic partners (i.e. plants) (Adler and Bronstein, 2004).

In predator-prey models with population structure, indirect effects along the trophic chain can produce very different dynamics from unstructured populations (Abrams and Quince, 2005; Roos et al., 2003a; Rudolf, 2007). For example, Rudolf (2007) found that behavioral interactions between predator stages (e.g. cannibalism) can alter the dynamics of predator-prey systems producing positive indirect effects that alter the strength of trophic cascades. In size-structured predator-prey populations, Roos et al. (2003b) found that if predators forage on specific prey sizes and prey have density-dependent individual development, an 'emergent Allee effect' is produced in the predators. This emergent Allee effect is due to a feedback of the predator density on its own performance and is modified by the life-history of the prey Roos et al. (2003a). Furthermore, organisms with complex life-cycles can mediate fluxes of energy between ecosystems (Schreiber and Rudolf, 2008), and early life-stages of pollinators have an important role determining population growth (Dempster, 1983; De Roos et al., 2007) implying that the consideration of population structure is extremely relevant when studying community dynamics. For example, some studies indicate that consumers with different life-stages can produce trophic cascades, affecting different ecosystems (Knight et al., 2008). Thus, we can expect different dynamics and stability conditions when considering population structure in plant-pollinator systems.

Here, we study a facultative-obligate plant-pollinator system with pollinator population structure and based on consumer-resource interactions (Holland and DeAngelis, 2010). This simple model assumes a more mechanistic plant-pollinator interaction (Soberon and Martínez del Río, 1981) than Lotka-Volterra models of mutualism (Addicott, 1981; Dean, 1983; May, 1976) by explicitly describing the resource and consumer dynamics between plants and pollinators, where there is an exchange of resources (i.e. nectar) for an ecological service (i.e. pollination). Our results indicate that population structure is highly important for the stability of plant-pollinator interactions and the management of pollination service.

THE MODELS

We consider two models of plant-pollinator interactions: model (I) with population structure and type I functional response and model (II) with population structure and type II functional response (Holling's disc equation). Model (II) is biologically more realistic, but the predictions are qualitatively similar to those of model (I), which is analytically more tractable.

The general structure of both models describes the dynamics of plants and their insect pollinators with a system of ordinary differential equations for the plant's biomass (P), a resource provided by the plants, such as nectar (N), and the biomass densities of adult insects (A) and their larvae (L). Pollination is modeled as a consumer-resource interaction in which nectar consumption is described by a functional response: $f(N, A)$. In the absence of insect pollination the plant population is able to grow according to the logistic model, but pollination by insects increases the growth rate in direct proportion to pollinator's functional response. The differential equations for plants and nectar are:

$$\frac{dP}{dt} = rP(1 - \delta_P P) + \sigma f(N, A) \tag{2.1}$$

$$\frac{dN}{dt} = \rho P - \delta_N N - f(N, A) \tag{2.2}$$

where in the first term in equation 2.1 r is the intrinsic growth rate and δ_P is a self-limitation coefficient, e.g. due to limiting nutrients. The second term accounts for the reproductive benefits from pollination through nectar consumption ($f(N, A)$), which depends on nectar and pollinator abundance. The parameter σ represents the pollination efficiency in terms of amount of plant biomass produced per nectar consumed, but it can also be taken as a proxy for the number of fertilized ovules per insect visit. Pollination efficiency can also be described by a plant trait (e.g. floral morphology), for example the anther exertion length, which determines the number of pollen removed by pollinators (Conner et al., 1995). Evidently, the benefits of pollination for the plant lie in increasing its equilibrium abundance (Addicott, 1981; Wolin and Lawlor, 1984). Nectar increases in proportion to plant biomass with production rate ρ, and decreases with a decay rate δ_N and with nectar consumption with rate α.

Insects use nectar to produce eggs from which larva emerge. Thus, the number of larva produced is directly proportional to the amount of nectar consumed. Only the adult stage exploits resources (i.e. nectar), implying that larvae do not interact with the plant. This could be the case for some Hymenopteran pollinators (e.g. honey bees), which spend their larval stage in nest cavities without interacting with plants directly (Roulston and Goodell, 2011) or pollinators that feed on different host plants in their larval and adult stages. The equations describing pollinator dynamics are:

$$\frac{dL}{dt} = \epsilon f(N, A) - \gamma L - \delta_L L \tag{2.3}$$

$$\frac{dA}{dt} = \gamma L - \delta_A A \tag{2.4}$$

where ϵ is the conversion efficiency for the transformation of nectar into larvae, γ is the per capita maturation rate and δ_L is the per capita larva mortality rate. Adult density increases by maturation of larvae and decreases by adult mortality at per capita rate δ_A.

Thus, the mutualistic interaction is assumed to be a facultative-obligatory system. Plants are facultative mutualists because they can survive and reproduce without the presence of pollinators, for example, through autogamy (i.e. self-fertilization) or clonal reproduction. However, insect pollinators are obligatory mutualists because they depend entirely on the consumption of nectar by the plants in order to produce larvae.

In model I, pollination is modeled as a consumer-resource interaction in which nectar consumption is proportional to the product of nectar times adult insect density, as in the linear type I functional response:

$$f(N, A) = \alpha N A \tag{2.5}$$

where α is the consumption rate per unit of nectar and per pollinator. Thus, pollination by insects increases the growth rate in direct proportion to nectar consumption rate α.

In model II, pollination is modelled with a type II functional response. Insect pollinators, like other consumers (e.g. herbivores), invest time in resource manipulation (i.e. handling time) (Ingvarsson and Lundberg, 1995; Herrera, 1989a). Thus, the pollination benefits for both plants and pollinators do not grow linearly, but in a saturating fashion. We can incorporate handling time in the consumer-resource interaction by using a type II functional response (Holling's disc equation) (Holling, 1959) in the model:

$$f(N, A) = \frac{\alpha N A}{1 + t_h \alpha N} \tag{2.6}$$

where t_h is the handling time of the pollinators.

In the Appendix we list the system parameters for both model alternatives (i and ii) together with the values employed for the numerical analysis.

ANALYSIS AND RESULTS

The analysis of the models consists of characterizing the equilibrium states $E = \{\hat{P}, \hat{N}, \hat{L}, \hat{A}\}$ and their stability. There are three classes of equilibrium states: the trivial equilibrium $E_0 = \{0, 0, 0, 0\}$ with plants and pollinators absent, the plant-only equilibrium $E_1 = \{\hat{P} > 0, \hat{N} > 0, \hat{L} = 0, \hat{A} = 0\}$ with the pollinators absent, and the plant-pollinator equilibrium with plants and pollinators present $E_2 = \{\hat{P} > 0, \hat{N} > 0, \hat{L} > 0, \hat{A} > 0\}$. Because $r > 0$, it immediately follows that E_0 is always unstable. The stability of E_1 and E_2 will be determined by the analysis of the eigenvalues of the Jacobian matrix of the system evaluated at E_1 and E_2 (details in the Appendix).

Model I

In the absence of the pollinators, the plants grow logistically and a plant-only equilibrium is attained: $E_1 = \{\hat{P} = \delta_P^{-1}, \hat{N} = \rho/(\delta_P \delta_N), \hat{L} = 0, \hat{A} = 0\}$. This equilibrium is unstable against invasion by a low number of animals, if and only if:

$$R_0 := \frac{\epsilon\alpha\rho\gamma}{\delta_P\delta_N\delta_A(\gamma+\delta_L)} > 1 \tag{2.7}$$

We call R_0 the pollinator basic reproduction ratio. It is the expected number of adults produced by one adult during its life-time. The rationale of Eq. 2.7 is as follows: from equation 2.3 the number of larvae produced by an average adult during an arbitrary time span Δt must be equal to $\epsilon\alpha N\Delta t$. During an invasion the amount of nectar available for the pollinators is $N = \rho/(\delta_P\delta_N)$, i.e. the equilibrium level when pollinators are absent. If the time span is the same as the life-span of an adult (i.e. $\Delta t = \delta_A^{-1}$), the average number of larvae produced by an adult during its life-time is $\epsilon\alpha\rho/(\delta_P\delta_N\delta_A)$. According to equation 2.3, the fraction of larvae that become adults is $\gamma/(\gamma+\delta_L)$ while the complement $\delta_L/(\gamma+\delta_L)$ dies. Thus, after one life-time cycle, 1 adult is replaced by $[\epsilon\alpha\rho/(\delta_P\delta_N\delta_A)] \times [\gamma/(\gamma+\delta_L)]$ new adults.

To obtain the plant-pollinator equilibrium E_2 we start by setting $dA/dt = 0$ in equation 2.4. This shows that the pollinator adult:larva ratio at E_2 is:

$$\frac{\hat{A}}{\hat{L}} = \frac{\gamma}{\delta_A} \tag{2.8}$$

i.e., the pollinator population structure depends on the larval maturation rate and the adult mortality rate. If maturation is fast relative to adult mortality ($\gamma \gg \delta_A$) the system will shift to a large proportion of adults versus larvae ($\hat{A} > \hat{L}$), and vice versa, slow maturation relative to adult mortality ($\delta_A \gg \gamma$) shifts the population towards a large proportion of larvae relative to adults ($\hat{L} > \hat{A}$). Equation 2.8 also tells us that R_0 is proportional to the adult:larva ratio, if $\delta_L \gg \gamma$, but in more general situations R_0 is just positively related with the adult:larva ratio.

We now set $dL/dt = 0$ in equation 2.3, where $\hat{L}$ and $\hat{A}$ can be eliminated using equation 2.8. This gives us the nectar equilibrium abundance:

$$\hat{N} = \frac{(\gamma+\delta_L)}{\epsilon\alpha} \times \frac{\delta_A}{\gamma} \tag{2.9}$$

For the plant abundance we combine equations 2.1 and 2.2 with $dP/dt = dN/dt = 0$. This results in a quadratic equation in $\hat{P}$, the solutions of which are:

$$\hat{P} = \frac{1+\omega}{2\delta_P}\left(1 \pm \sqrt{1 - \frac{4\omega}{(1+\omega)^2 R_0}}\right) \tag{2.10}$$

where $\omega = \frac{\sigma\rho}{r}$ is a compound parameter which will turn out to play a key role. By substituting this in equation 2.1 with $dP/dt = 0$ we can obtain the adult density, and the larval equilibrium follows from equation 2.8:

$$\hat{A} = \frac{\rho\hat{P}(\delta_P\hat{P}-1)}{\omega\alpha\hat{N}} \tag{2.11}$$

$$\hat{L} = \frac{\rho\hat{P}(\delta_P\hat{P}-1)}{\omega\alpha\hat{N}} \times \frac{\delta_A}{\gamma} \tag{2.12}$$

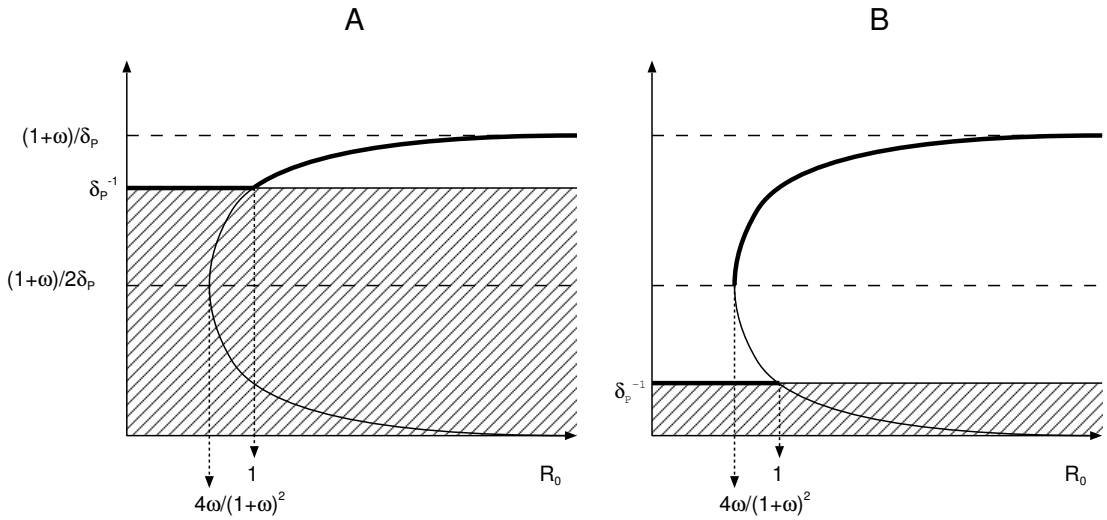

Figure 2.1: Plant equilibrium densities as a function of animal basic reproductive ratio R_0in model type I. The horizontal line at $\hat{P} = \delta_P^{-1}$ corresponds to the plant-only equilibrium, which is locally stable for $R_0 \leqslant 1$ and unstable for $R_0 > 1$. The plant-animal equilibria are represented by a curve starting with two symmetric branches $\hat{P}_{HI}$ and $\hat{P}_{LO}$ above and below $P = \frac{1+\omega}{r\delta_P}$ respectively. The upper branch $\hat{P}_{HI}$ corresponds to the plant-animal mutualism, and is stable (numerically determined); the lower branch $\hat{P}_{LO}$ is unstable and corresponds to a saddle point. Equilibrium values in the hatched region are unfeasible (i.e. they correspond with negative pollinator densities). (A) If $\sigma\rho < 1$ the system shows mutualism for $R_0 > 1$ without Allee effect. (B) If $\omega > 1$ the system shows mutualism with Allee effect for $\frac{4\omega}{(1+\omega)^2} < R_0 < 1$ and without Allee effect for $R_0 > 1$.

These are biologically feasible only if they are real numbers and positive. The solution of 2.10 (and by extension 2.11,2.12) is real if and only if :

$$R_0 \geqslant \frac{4\omega}{(1+\omega)^2} \tag{2.13}$$

Under this condition, the square root in Eq. 2.10 is less than or equal to 1. If the inequality holds, $\hat{P}$ exists as a real-valued pair $(\hat{P}_{HI}, \hat{P}_{LO})$ corresponding to the "+" and "-" cases in equation 2.10. Hence, $\hat{A}$ and $\hat{L}$ also exist as pairs $(\hat{A}_{HI}, \hat{A}_{LO})$ and $(\hat{L}_{HI}, \hat{L}_{LO})$ respectively. Thus, the plant-pollinator mutualism can involve two real equilibria $E_{2,HI} = (\hat{P}_{HI}, \hat{N}, \hat{L}_{HI}, \hat{A}_{HI})$ and $E_{2,lo} = (\hat{P}_{LO}, \hat{N}, \hat{L}_{LO}, \hat{A}_{LO})$. If the equality holds in 2.13 (a saddle-node bifurcation point), the two equilibria coincide. In equations 2.11 and 2.12, we see that $E_{2,HI}$ or $E_{2,LO}$ will be biologically feasible if and only if $\hat{P}_{HI}$ or $\hat{P}_{LO}$, respectively, are larger than δ_P^{-1}, which is the plant equilibrium in the absence of the mutualism. In Figure 2.1 we sketch the plant equilibrium abundance (graph of 2.10) as a function of the pollinator's R_0, to illustrate the feasibility conditions of the mutualistic equilibrium.

In Figure 2.1 we can see that if the pollinator is able to invade when rare $(R_0 > 1)$ there will always be a single feasible plant-pollinator equilibrium $(E_{2,HI})$. If the pollinator is not

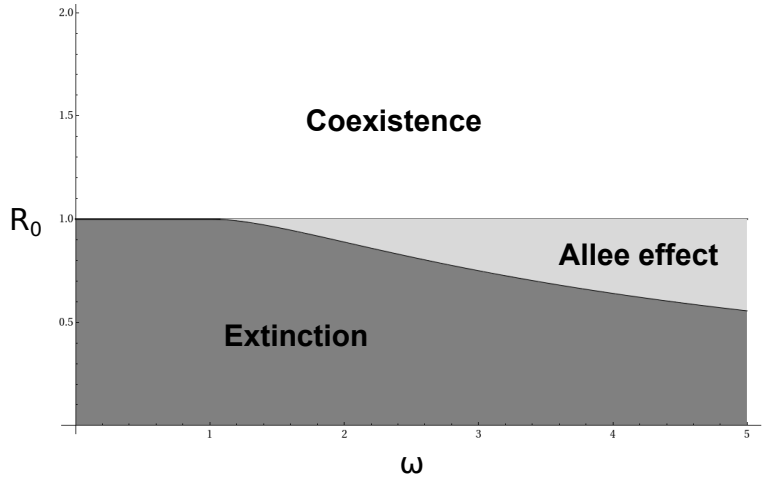

Figure 2.2: Parameter space plot of the plant-pollinator mutualism model type I, animal basic reproductive ratio (R_0) vs. plant's mutualistic offset (ω). The parameter space is divided into three regions of coexistence and stability: i) *Pollinator extinction*: $R_0 < 1$ for $\omega < 1$ and $R_0 < \frac{4\omega}{(1+\omega)^2}$ for $\omega > 1$; this is the region where animal pollinators cannot survive under any condition and consequently the mutualism is not possible. ii) *Allee effect*: $\frac{4r\sigma\rho}{(1+\omega)^2} < R_0 < 1$; this is the Allee effect area for animal pollinators, which increases with the plant's mutualistic offset ($\omega > 1$). This region is unstable for the plant-pollinator mutualism, only pollinators above the extinction threshold can survive. iii) *Plant-pollinator coexistence*: $R_0 > 1$. In this region, the plant-pollinator mutualism is globally stable. For parameters values used look at the Appendix .

able to invade when rare, the two plant-pollinator equilibria ($E_{2,HI}$ and $E_{2,LO}$) are feasible if R_0 satisfies:

$$\frac{4\omega}{(1+\omega)^2} < R_0 < 1 \tag{2.14}$$

We call the compounded parameter $\omega = \sigma\rho/r$, the plant's mutualistic offset ω. The fact that whenever there is a single feasible plant-pollinator equilibrium the pollinator is always able to invade, and that whenever there are two plant-pollinator equilibria the pollinator cannot invade, suggests the existence of a strong Allee effect, like in other models with at least one obligate mutualist partner (Wilson et al., 2003; Holland, 2002; Vandermeer and Boucher, 1978; Soberon and Martínez del Río, 1981). With numerical stability analyses (see Appendix), we determined that if $\omega > 1$ equilibrium $E_{2,HI}$ is always locally stable and $E_{2,LO}$ is always unstable, i.e. $E_{2,LO}$ must be an extinction and invasion threshold for the pollinator. If $\omega < 1$ equilibrium $E_{2,HI}$ is only locally stable for $R_0 > 1$.

Summarizing, from the graphical analysis of Figure 2.1 and local stability conditions, we can classify three different mutualistic regimes:

$$\omega \begin{cases} < 1 & \text{mutualism without Allee effect when: } R_0 > 1 \\ > 1 & \text{mutualism} \begin{cases} \text{without Allee effect when:} & R_0 > 1 \\ \text{with Allee effect when:} & \frac{4\omega}{(1+\omega)^2} < R_0 < 1 \end{cases} \end{cases} \tag{2.15}$$

The Allee effect occurs under ecological scenarios in which plant's mutualistic offset (ω) is greater than 1. This occurs when its effect of mutualism on the pollination efficiency (σ) mutiplied by the rate of nectar production (ρ) is larger than the plant's intrinsic growth rate (r), that is, when mutualism is relatively more important for the plant demographics compared to growing without the interaction. This is illustrated by Figure 2.2, illustrating the stability and pollinator invasion boundary as well as parameter conditions that cause the Allee effect.

As we mentioned previously, R_0 is partly determined by the population structure: R_0 increases when the adult:larva ratio increases by larger maturation rates and lower adult mortality. This relationship between R_0 and the animal population structure will ultimately affect the amount of benefits received by the plants from pollinators; i.e. plant benefits from pollination will be high if there is a large proportion of adults to larvae ($\gamma \gg \delta_A$), pollination efficiency is high ($\sigma \gg 1$). However, even when reproduction by pollination efficiency is high ($\sigma \gg 1$) and a change in the population structure with a small proportion of adults to larvae ($\gamma \ll \delta_A$) could bring pollinators to the region where the Allee effect jeopardizes the stability of the plant-animal interaction, as shown in Figure 2.2. The combined effect of pollinator population structure (γ/δ_A) and high plant's mutualistic offset (ω) in plant densities can also be seen in Figure 2.3: when pollinator population structure is dominated by adults ($\gamma/\delta_A > 1$), plant equilibrium densities rapidly increase when there is a stronger efficiency and dependence on pollination service ($\omega > 1$). Finally, an increase in pollination service ($\omega > 1$) puts the pollinator population in the Allee effect region when $\gamma/\delta_A < 1$, creating the possibility of a catastrophic collapse of the mutualism.

Model II

The model with type II functional response exhibits the same qualitative behavior with respect to stability and coexistence of plant-pollinator mutualism as model I (see Appendix for details). The condition of pollinator growth when rare in this model is that the basic reproduction ratio is again higher than 1:

$$R_0 = \frac{\epsilon \rho \alpha \gamma}{\delta_A(\delta_P \delta_N + t_h \alpha \rho)(\gamma + \delta_L)} > 1 \tag{2.16}$$

The main difference between both models is related to the effect of pollinator's handling time, as can be seen in the basic reproductive ratio (equation 2.16). An increase in handling time produces a saturating effect in the pollination service and the equilibrium density of the animals. The condition for the Allee effect (eq. 2.13) is exactly the same as in model I and the stability conditions for the plant-pollinator coexistence are qualitatively similar to the previous model (see Appendix). Interestingly, pollinators with larger handling times ($t_h \gg 0$) and therefore relatively low R_0 are able to exist in the Allee effect region as long as pollination service is highly efficient ($\omega > 1$). This is because there is no relationship

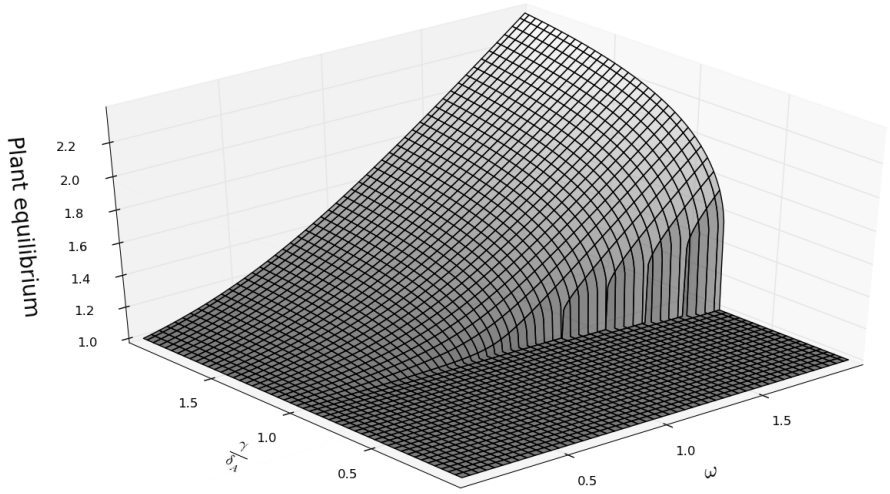

Figure 2.3: The effect of pollinator population structure on the plant equilibrium in model I. Changes along the γ/δ_A axis represent a shift in pollinator population structure. If $\gamma/\delta_A < 1$, the pollinator population is dominated by larvae, however if $\gamma/\delta_A > 1$, the pollinator population structure is dominated by adults. The increase of plant equilibrium densities depends on the population structure and the plant's mutualistic offset (ω). The sudden jump in the figure starting when $\omega > 1$ and $\gamma/\delta_A < 1$ shows the critical transition between alternative stable states (i.e. bistability) of the Allee effect region. For parameters values used look at the Appendix.

between handling time (t_h) and pollination efficiency (σ) . R_0 is only affected by t_h (eq. 2.16) while the lower bound to R_0 is only affected by σ. Thus, short time visits to flowers pollinating a large number of flowers can be viable as well as long time visits with efficient pollination of only few flowers.

DISCUSSION

Determining the stability of the mutualistic interaction has been the main interest of classical theoretical studies. May (1976) found that obligate mutualistic interactions are very unstable and prone to extinction. Later, several studies showed that mutualism can be stable when intraspecific competition is strong relative to the mutualistic interaction (Dean, 1983; Addicott, 1981). Addicott (1981) argued that if mutualistic interaction coefficients are decreasing functions of density (Vandermeer and Boucher, 1978), then a locally unstable equilibrium does not necessarily imply that the system is globally unstable (Travis and Post, 1979).

Recently, theoretical research has mainly focused on more mechanistic models of obligatory plant-pollinator interactions (e.g. fig-fig wasp) on eco-evolutionary dynamics (Ferriere et al., 2007; Ferdy et al., 2002) and on other types of ecological interactions mediating mutualism (Bronstein et al., 2006); for example, antagonistic interactions (e.g. herbivores, parasites) in mutualistic systems (Wilson et al., 2003; Bronstein et al., 2003b) can make mutualism more unstable and prone to extinction under certain conditions.

However, previous models have ignored pollinator population structure as a crucial component of the stability of plant-pollinator interactions. Our model shows that pollinator population structure is important for the stability and conservation of plant-pollinator interactions. Specifically, we find that decreases in larval maturation rate relative to adult mortality shifts the pollinator population towards a larvae dominated population decreasing the pollination service and jeopardizing the interaction.

The question is what could cause such a detrimental effect in plant-pollinator interactions. The current global pollinator decline, particularly specialist bees (i.e. oligolectic bees) (Larsson and Franzén, 2007; Biesmeijer et al., 2006), has stimulated research aiming at understanding the multiple causes that impair pollinator's population growth (Kearns et al., 1998; Potts et al., 2010). Apart from natural pathogens ((Pettis et al., 2012a)), pesticides are among the most important causes, slowing the larval maturation rate and increasing the adult mortality rate, particularly in Hymenopteran pollinators (Wu et al., 2011; Roulston and Goodell, 2011; Krupke et al., 2012). Pesticides have various negative effects in the survivorship and development of bee colonies: they can impair foraging behavior, decrease egg production, delay larval development and shorten adult longevity (Wu et al., 2011; Roulston and Goodell, 2011; Krupke et al., 2012; Pettis et al., 2012a; Morandin and Winston, 2003). Our model predicts that these effects of pesticides can produce a shift in the pollinator population structure to higher larva to adult ratios and decrease the population growth ($R_0 < 1$) putting the pollinators in the Allee effect region (i.e. bistability region). Furthermore, due to hysteresis (Scheffer and Carpenter, 2003), after a perturbation a pollinator population that was close to the fold bifurcation point (i.e. critical transition (Scheffer et al., 2009)) will not recover by, for example, an increase of nectar production rate (ρ) to the values where the transition occurred, i.e. it will not return to the alternative stable state of coexistence with plants (E_{HI}). Such a return requires a large increase in pollinator abundance that cannot be achieved by restoring the nectar production rate alone. This has important consequences for the management of pollination service in crop-pollinated fields because these critical transitions might be detectable before the population collapses (Scheffer et al., 2009).

Our model only explores the dynamics between a facultative plant and an obligate pollinator. That is, strictly speaking we only investigate a case of specialist pollinators, such as oligolectic bees. However, this type of pollinators are at a higher risk of collapse (Biesmeijer et al., 2006). Furthermore, our model allows to draw some conclusions also in the case of generalist pollinators, such as honey bees (Zayed et al., 2005; Biesmeijer et al., 2006). Honey bees, which often depend on a limited number of pollen/nectar resources because of habitat fragmentation (Kremen et al., 2002; Roulston and Goodell, 2011; Franzén and Nilsson, 2009) or suffer from a reduction in larval maturation rate due to pesticides (Wu et al., 2011), show the same catastrophic consequences as specialist pollinators. Thus, we believe that our results are relevant for plant-pollinator systems in general. Our model

only studied a pair-wise interaction and not a community. Although simple models provide much insight, it is essential that in future theoretical studies we incorporate population structure into mutualistic community dynamics models ((Bastolla et al., 2009; Okuyama and Holland, 2008)) to generate predictions for the management of pollination services and conservation of threatened species. We also advocate the future consideration of models that consider the conflict between mutualistic and antagonistic effects from different pollinator life-stages on the plants. This is particularly common in Lepidopteran pollinators (Adler and Bronstein, 2004; Kessler et al., 2010)

Adding a nectar handling time does not change qualitatively the conditions for an Allee effect, but it quantitatively directly affects the stability of the mutualism, as has been found in other models (Soberon and Martínez del Río, 1981; Ingvarsson and Lundberg, 1995). Increases in handling time decrease the pollinator basic reproductive ratio (R_0); hence longer handling times will drive pollinators to extinction or to the Allee effect region if pollination efficiency is high enough (see condition 2.14). In our model, pollination efficiency is independent of the pollinator's handling time. Thus, in the Allee effect region we can find 'slow' pollinators if there is high pollination efficiency. Several studies have found a negative correlation between pollination efficiency and handling time (Pattersson, 1991; Mitchell and Waser, 1992). Other studies report that pollination efficiency and handling time can be positively correlated (Conner et al., 1995; Ivey et al., 2003; Thomson, 1986). These differences seem to depend on the plant and pollinator species studied and the components of pollination efficiency measured (Herrera, 1989a; Ivey et al., 2003). For the plants, there is a clear advantage in having an efficient pollination service and different floral traits might evolve to increase flower-handling time (e.g. evolution of flexible pedicels (Hurlbert et al., 1996)), but stability of this interaction essentially will depend on the cost-benefit balance (Holland, 2002) and the community context (i.e. structure and composition of the community) (Okuyama and Holland, 2008).

We conclude that population structure is crucial for the stability of plant-pollinator interactions. The inclusion of population, temporal (i.e. phenology) and spatial structure is fundamental to properly conserve and manage plant-pollinator communities.

ACKNOWLEDGEMENTS

We thank David Alonso for helping in earlier versions of this model.

APPENDIX

Analysis of model with type I functional response

Model I has the following set of equations:

$$\frac{dP}{dt} = rP(1-\delta_P P) + \sigma\alpha NA \tag{2.17}$$
$$\frac{dF}{dt} = \rho P - \delta_N N - \alpha NA \tag{2.18}$$
$$\frac{dL}{dt} = \epsilon\alpha NA - \gamma L - \delta_L L \tag{2.19}$$
$$\frac{dA}{dt} = \gamma L - \delta_A A \tag{2.20}$$

An equilibrium is denoted as $E = \{\hat{P}, \hat{N}, \hat{L}, \hat{A}\}$. Since $r > 0$, the trivial equilibrium $E_0 = \{0, 0, 0, 0\}$ is always unstable, because for any arbitrarily small population, the plants will increase exponentially. In the absence of the pollinator, the plant population eventually converges to the plant-only equilibrium $E_1 = (\delta_P^{-1}, \rho(\delta_P\delta_N)^{-1}, 0, 0)$. The Jacobian matrix of the system evaluated at E_1 is:

$$J = \begin{bmatrix} -r & 0 & 0 & \frac{\sigma\alpha\rho}{\delta_P\delta_N} \\ \rho & -\delta_N & 0 & -\frac{\alpha\rho}{\delta_P\delta_N} \\ 0 & 0 & -(\gamma+\delta_L) & \frac{\epsilon\alpha\rho}{\delta_P\delta_N} \\ 0 & 0 & \gamma & -\delta_A \end{bmatrix}$$

and its eigenvalues are the eigenvalues of J are the eigenvalues of $J_p = \begin{bmatrix} -r & 0 \\ \rho & -\delta_N \end{bmatrix}$ and $J_a = \begin{bmatrix} -(\gamma+\delta_L) & \frac{\epsilon\alpha\rho}{\delta_P\delta_N} \\ \gamma & -\delta_A \end{bmatrix}$. The matrix J_p accounts for the effects of perturbations of the plant population on its own stability, i.e. its the internal stability. The matrix J_a accounts for the effects of perturbations on the plant-equilibrium caused by the invasion of small numbers of pollinators. The eigenvalues of J_p are $-r$ and $-\delta_N$ which means that the plant population is internally stable as would be expected since it grows logistically and flowers decay spontaneously. Thus, the system can only be externally de-stabilized by invasion when J_{12} has eigenvalues with positive real parts. The eigenvalues of J_{12} are:

$$\lambda = \frac{1}{2}\left(-(\gamma+\delta_L+\delta_A) \pm \sqrt{(\gamma+\delta_L+\delta_A)^2 - 4\left((\gamma+\delta_L)\delta_A - \frac{\epsilon\alpha\rho\gamma}{\delta_P\delta_N}\right)}\right)$$

So E_1 is unstable only if:

$$\frac{\epsilon\alpha\rho\gamma}{\delta_P\delta_N} > (\gamma+\delta_L)\delta_A$$

which can be also written as the pollinator invasion condition in terms of the basic reproduction ratio R_0:

$$R_0 = \frac{\epsilon\alpha\rho\gamma}{\delta_P\delta_N(\gamma+\delta_L)\delta_A} > 1 \tag{2.21}$$

The local stability of the plant-pollinator equilibrium E_2 depends on the eigenvalues of the Jacobian matrix of the system evaluated at E_2 (recall that E_2 consists of two equilibrium branches as shown in the main text):

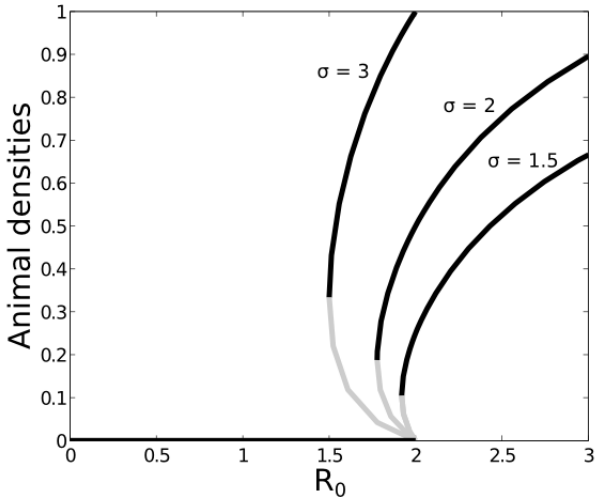

Figure 2.4: Bifurcation of the plant-pollinator system, displaying the adult pollinator densities in the plant-pollinator (curves) as well as the plant-only equilibrium (the $A = 0$ line) branches. The parameter axis uses α which is a direct proxy of R_0. Black coloring correspond to stable equilibria and grey coloring to unstable equilibria. The plots are done for several values of the σ, showing a positive effect on pollinator equilibrium densities, but no effect on the its invasion requirement (because R_0 is independent of σ)

$$J = \begin{bmatrix} r(1-2\delta_P\hat{P}) & \sigma\alpha\hat{A} & 0 & \sigma\alpha\hat{N} \\ \rho & -(\delta_F+\alpha\hat{A}) & 0 & -\alpha\hat{N} \\ 0 & \epsilon\alpha\hat{A} & -(\gamma+\delta_L) & \epsilon\alpha\hat{N} \\ 0 & 0 & \gamma & -\delta_A \end{bmatrix} \tag{2.22}$$

The characteristic equation of (2.22) cannot be factored in a simple fashion that let us infer the signs of its eigenvalues. Therefore, we evaluated the local stability of the plant-animal equilibrium system by numerical bifurcation analysis of the two equilibrium branches $E_{2,hi}$ and $E_{2,lo}$ using the program XPPAUT (Ermentrout, 2002). In the main text we depict the dependence of the $E_{2,hi}$ and $E_{2,lo}$ branches as a function of R_0, which is itself a combination of many parameters. Here in Figure 2.4 we display the bifurcation of equilibria as a function of the pollinator basic reproduction ratio R_0. We plotted adult insects instead of the plant's equilibrium; in either case we see the same pattern in which the stable $E_{2,hi}$ and unstable $E_{2,lo}$ branches collide and annihilate as a saddle-node bifurcation.

ANALYSIS OF MODEL WITH TYPE II FUNCTIONAL RESPONSE

Model II has the following set of equations:

$$\frac{dP}{dt} = rP(1-\delta_P P) + \frac{\sigma\alpha NA}{1+t_h\alpha N} \tag{2.23}$$

$$\frac{dN}{dt} = \rho P - \delta_N N - \frac{\alpha NA}{1+t_h\alpha N} \tag{2.24}$$

$$\frac{dL}{dt} = \frac{\epsilon\alpha NA}{1+t_h\alpha N} - \gamma L - \delta_L L \tag{2.25}$$

$$\frac{dA}{dt} = \gamma L - \delta_A A \tag{2.26}$$

An equilibrium is denoted again as $E = \{\hat{P}, \hat{N}, \hat{L}, \hat{A}\}$. The trivial equilibrium $E_0 = \{0,0,0,0\}$ is always unstable for the same reasons as in model I. A small amount of plants will grow and attain an equilibrium $E_1 = \{\delta_P^{-1}, \rho(\delta_P\delta_F)^{-1}, 0, 0\}$. The Jacobian matrix at E_1 is:

$$J = \begin{bmatrix} -r & 0 & 0 & \frac{\sigma\alpha\rho}{\delta_P\delta_N+t_h\alpha\rho} \\ \rho & -\delta_N & 0 & -\frac{\alpha\rho}{\delta_P\delta_N+t_h\alpha\rho} \\ 0 & 0 & -(\gamma+\delta_L) & \frac{\epsilon\alpha\rho}{\delta_P\delta_N+t_h\alpha\rho} \\ 0 & 0 & \gamma & -\delta_A \end{bmatrix}$$

The eigenvalues of J_1 are the eigenvalues of $J_p = \begin{bmatrix} -r & 0 \\ \rho & -\delta_N \end{bmatrix}$ and $J_a = \begin{bmatrix} -(\gamma+\delta_L) & \frac{\epsilon\alpha\rho}{\delta_P\delta_N+t_h\alpha\rho} \\ \gamma & -\delta_A \end{bmatrix}$. Like before the eigenvalues of J_p are $-r$ and $-\delta_N$, and E_1 is internally stable. The two eigenvalues of J_a are:

$$\lambda = \frac{1}{2}\left(-(\gamma+\delta_L+\delta_A) \pm \sqrt{(\gamma+\delta_L+\delta_A)^2 - 4\left((\gamma+\delta_L)\delta_A - \frac{\epsilon\alpha\rho}{\delta_P\delta_N+t_h\alpha\rho}\right)}\right)$$

so E_1 is (externally) unstable if λ has positive real parts, which happens if and only if:

$$\frac{\epsilon\alpha\rho\gamma}{\delta_P\delta_N+t_h\alpha\rho} > (\gamma+\delta_L)\delta_A$$

a condition that can be written as the pollinator invasion condition:

$$R_0 = \frac{\epsilon\alpha\rho\gamma}{(\delta_P\delta_N+t_h\alpha\rho)(\gamma+\delta_L)\delta_A} > 1 \tag{2.27}$$

As in the main text, R_0 is the basic reproduction ratio because it can be factored as follows:

$$R_0 = \frac{\alpha(\rho/\delta_P\delta_N)}{1+t_h\alpha(\rho/\delta_P\delta_N)} \times \frac{\gamma}{\gamma+\delta_L} \times \frac{1}{\delta_A}$$

where the first factor would be the number of larvae produced per adult in a plant-only equilibrium, when nectar abundance is $\rho/\delta_P\delta_N$, the second factor is the proportion of larvae than avoid death and become adults, and the third factor is the average adult life-time. Thus R_0 is the number of adults that replace an adult when it dies.

Appendix

As in model I, in the plant-animal equilibrium E_2 the adult pollinator equation 2.20 leads to a fixed stage-structure:

$$\frac{\hat{A}}{\hat{L}} = \frac{\gamma}{\delta_A} \tag{2.28}$$

which by substitution in the larvae equation 2.25 leads to the nectar equilibrium:

$$\hat{N} = \frac{(\gamma + \delta_L)\delta_A}{\epsilon\alpha\gamma - \delta_A t_h \alpha(\gamma + \delta_L)} \tag{2.29}$$

To obtain the plant density in E_2 notice that the equilibrium conditions of plants and nectar in equations (2.17,2.18) share the common term $\frac{\alpha\hat{N}\hat{A}}{1+t_h\alpha\hat{N}}$ resulting in two equations:

$$\frac{\alpha N A}{1 + t_h \alpha N} = -\frac{\rho P(1 - \delta_P P)}{\omega} \tag{2.30}$$

$$\frac{\alpha N A}{1 + t_h \alpha N} = \rho P - \delta_N N \tag{2.31}$$

which can be combined into a second degree equation in $\hat{P}$. After substituting $\hat{N}$ by 2.29, the equation can be solved for $\hat{P}$ as:

$$\hat{P} = \frac{1 + \omega}{2\delta_P}\left(1 \pm \sqrt{1 - \frac{4\omega}{(1+\omega)^2 R_0}}\right) \tag{2.32}$$

This is the same result as in model I, only that the definition for R_0 in model II differs by the inclusion of the handling time in 2.27. There are two solutions corresponding to $\hat{P}_{hi}$ and $\hat{P}_{lo}$ corresponding to the "+" and "-" signs in 2.32 respectively. It follows then from the plant equation and from the constant structure relationship that

$$\hat{A} = \frac{\rho}{\omega} \times \frac{\hat{P}(\delta_P \hat{P} - 1)(1 + t_h \alpha \hat{N})}{\alpha \hat{N}}, \hat{L} = \frac{\rho}{\omega} \times \frac{\hat{P}(\delta_P \hat{P} - 1)(1 + t_h \alpha \hat{N})\delta_A}{\alpha\gamma\hat{N}}$$

will have "hi" and "lo" values corresponding to $\hat{P}_{hi}$ and $\hat{P}_{lo}$ and that E_2 can consist of two equilibria $E_{2,hi} = (\hat{P}_{hi}, \hat{N}, \hat{L}_{hi}, \hat{A}_{hi})$ and $E_{2,lo} = (\hat{P}_{lo}, \hat{N}, \hat{L}_{lo}, \hat{A}_{lo})$. The feasibility of which will depend again on $\hat{P}$ in 2.32 being real valued, and of $\hat{P}$ being higher than δ_P^{-1}, which are the same conditions as in model I (only the definition of R_0 differ).

The local stability of the equilibrium E_2 depends on the eigenvalues of the Jacobian matrix of the system evaluated at E_2:

$$J = \begin{bmatrix} r(1 - 2\delta_P \hat{P}) & \frac{\sigma\alpha\hat{A}}{(1+t_h\alpha\hat{N})^2} & 0 & \frac{\sigma\alpha\hat{N}}{1+t_h\alpha\hat{N}} \\ \rho & -(\delta_N + \frac{\sigma\alpha\hat{A}_{\pm}}{(1+t_h\alpha\hat{N})^2}) & 0 & -\frac{\alpha\hat{N}}{1+t_h\alpha\hat{N}} \\ 0 & \frac{\epsilon\alpha\hat{A}}{(1+t_h\alpha\hat{N})^2} & -(\gamma + \delta_L) & \frac{\epsilon\alpha\hat{N}}{1+t_h\alpha\hat{N}} \\ 0 & 0 & \gamma & -\delta_A \end{bmatrix} \tag{2.33}$$

This Jacobian matrix is again not suitable for direct analysis, so we applied again a bifurcation analysis to asses the stability of E_2. We performed the analysis in terms of the

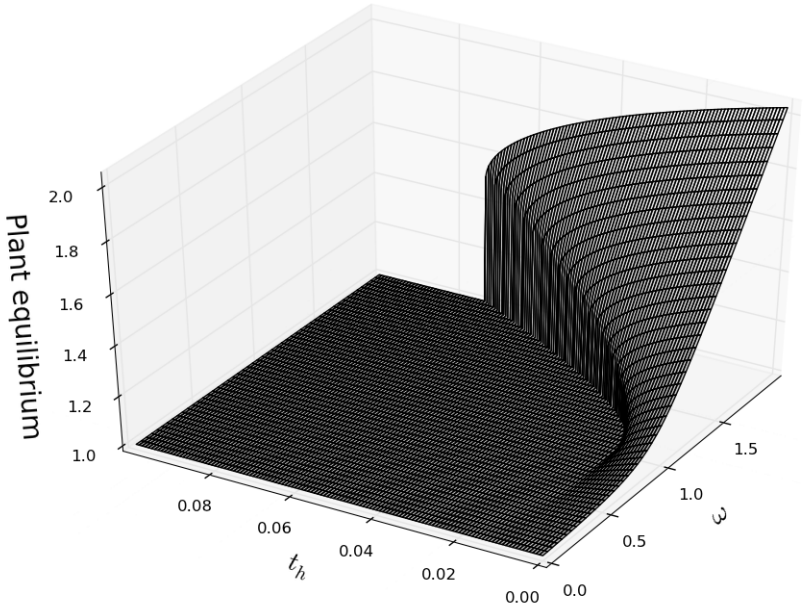

Figure 2.5: Effect of handling time (t_h) and plant's mutualistic offset (ω) on plant equilibrium densities. The relationship between handling time and pollination service is non-linear, with increases in handling time the Allee effect region increases producing higher $E_{2,hi}$ equilibrium values. The vertical wall in the figure starting when $\omega > 0.5$ and $t_h > 0.01$ shows the critical transition between alternative stable states (i.e. bistability) of the Allee effect region. Parameters used: $r = 1$, $\delta_P = 1$, $\rho = 1$, $\delta_N = 0.49$, $\epsilon = 1$, $\alpha = 1$, $\delta_L = 1$, $\gamma = 1$, $\delta_A = 1$.

nectar consumption rate like for model I, obtaining a qualitatively identical result, i.e. when feasible, the higher equilibrium branch ($E_{2,hi}$) is always stable and the lower ($E_{2,lo}$) always unstable, and both branches collide and annihilate in a saddle-node bifurcation. In this appendix we show instead in Figure 2.5 the effect of handling time t_h and plant's mutualistic offset $\omega = \sigma\rho/r$ on plant equilibrium density.

Table 2.1: Model parameters

Name	*Symbol*	*Units*	*Values*
Densities	P, N, L, A	$mass \times area^{-1}$	
intrinsic growth rate	r	$time^{-1}$	1
plant density dependence	δ_P	$(mass \times time)^{-1}$	1
nectar production rate	ρ	$time^{-1}$	1
nectar decay	δ_N	$time^{-1}$	0.49
attack rate	α	$mass^{-1}$	1
pollination efficiency	σ	—	
pollinator conversion factor	ϵ	—	1
larval maturation rate	γ	$time^{-1}$	1
larval mortality	δ_L	$time^{-1}$	1
adult mortality	δ_A	$time^{-1}$	0.9
handling time	t_h		1
plant's mutualistic offset	ω		1

3

FRUGIVORE BEHAVIOR MAKES FRUITING FRUITFUL

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Animal seed dispersal provides an important ecosystem service by strongly benefitting plant communities. There are several theoretical studies on the ecology of plant-animal seed-disperser interactions, but few studies have explored the evolution of this mutualism. Moreover, these studies ignore plant life-history, and frugivore foraging behavior. Thus, it remains an open question what the conditions for the diversification of fruit traits are, in spite of the multitude of empirical studies on fruit trait diversity. Here we study the evolution of fruit traits using a spatially-explicit individual-based model, which considers the costs associated with adaptations inducing dispersal by frugivory, as well as frugivore foraging behavior and abundance. Our model predicts that these costs are the main determinants of the evolution of fruit traits, and that when the costs are not very high, the evolution of larger fruit traits (e.g. fleshy/colorful fruits) is controlled by the choosiness and response thresholds of the frugivores as well as their numerical abundance.

KEYWORDS: frugivory, endozoochory, seed dispersal, fruit traits, fruit evolution, mutualism

INTRODUCTION

The survival and reproduction of most angiosperm plants highly depend on the ecological dispersal service provided by frugivorous animals (Janzen, 1970; Connell, 1971; Howe and Smallwood, 1982b; **?**). Around 90% of tropical tree species produce fleshy fruits dispersed by vertebrate animals, such as mammals and birds (**?**). Frugivores consume fruits produced by the plants and actively disperse their seeds over long distances (Howe and Smallwood, 1982b). In this study, we focus on the most widespread biotic dispersal syndrome, endozoochory, where frugivores regurgitate, defecate and release the seeds in more favorable environments, while benefitting themselves from the energy and nutrients of the fruits (**?**). This mutualistic interaction seems to be responsible for the establishment and radiation of angiosperm plants in terrestrial ecosystems (Howe and Smallwood, 1982b; **?**; **?**). Frugivores thus represent a predominant selective force on the evolution of flowering plants (Jordano, 1987).

The "dispersal syndrome" hypothesis argues that evolutionary convergence of fruit traits (e.g. color, size, aromas, nutrients) in different plant species is driven by a set of similar frugivorous species (**??**). Therefore, the diversification of fruit traits might be the outcome of different selective pressures from frugivores with different visual/olfactory perceptions (Schaefer et al., 2007, 2008; Schaefer and Schmidt, 2004; Valido et al., 2011; Kalko and Condon, 1998), social behavior (Russo et al., 2006; Russo and Augspurger, 2004; Howe, 1989) and/or morphology (e.g. gape width) (**?**Flörchinger et al., 2010). However, little is still known about what ecological conditions and evolutionary forces drive the diversification of fruit traits (Bolmgren and Eriksson, 2010; **?**). Several studies support the dispersal syndrome hypothesis (**?**Gautier-Hion et al., 1985; **?**) and others reject it (**?**). Furthermore, there are many empirical studies on fruit diversification stating different hypotheses and predictions that have not been considered in the theoretical literature (Willson and Whelan, 1990; Schaefer et al., 2007; **?**; Valido et al., 2011). Thus, it remains an open question whether seed dispersal syndromes can explain the evolution of fruit diversity (i.e. diversity in terms of size, color, nutrient content) (Flörchinger et al., 2010).

To our knowledge, there are few mathematical models specifically dealing with the evolution of animal seed-dispersal. There are models that explain the evolution of seed dispersal kernels (Hovestad et al., 2001; Starrfelt and Kokko, 2010), but they do not consider animal induced dispersal. Moreover, most mathematical models do not consider trade-offs affecting plant investments in traits promoting frugivory, nor, even more importantly, the consequences of animal behavior for the quality of the dispersal service. In summary, key features of this mutualistic interaction remain unexplored in theoretical studies investigating their evolutionary dynamics, in spite of their importance for the ecology of angiosperm plants (**?**) and evolution of fruit traits (Valido et al., 2011).

In this paper we will investigate the evolution of fruit traits involved in frugivory and dispersal by endozoochory. For this we will use a simulation model incorporating key aspects of plant life-history. This will allow us to assess the effect of different life-cycle parameters on population viability, and to determine under which conditions frugivory benefits plants and fruit traits can evolve. Our model combines three important features not considered together in previous models. First, by considering the plant's life-cycle we can study the effect of trade-offs related to fruit production costs on plant fitness. Second, the

mutualism is treated as a consumer-resource interaction, where the extent of consumption affects the benefits for the plants. And third, it accounts for the effect of density-dependence in the cost-benefit balance for the plants. We will study the evolution of endozoochorous adaptations, i.e. metric traits that induce or facilitate frugivory, such as fruit size, fruit pigmentation, chemical attractants, etc. (Howe and Smallwood, 1982b; Gautier-Hion et al., 1985; Willson and Whelan, 1990). On the one hand, the investment in such traits has costs and leads to trade-offs (Eriksson and Jakobsson, 1999; Alcántara and Rey, 2003; Pakeman and Small, 2009). On the other hand, aspects of frugivore behavior, such as choosiness and the threshold to respond to fruit traits , and seed release patterns, will determine whether such investments contribute to plant fitness (Russo et al., 2006).

MODEL AND METHODS

Statement of the problem

Consider a fruit-producing species. There are three paths on the plant's life cycle that cause population changes from one year to the next: survival of adult trees (path "0"), recruitment from fruits not consumed by frugivores (path "1") and recruitment from fruits consumed by frugivores (path "2"). The plant's growth rate would be:

$$R = \underbrace{p}_{\text{"path 0"}} + f\Big[\underbrace{(1-c)g_1}_{\text{"path 1"}} + \underbrace{c\epsilon g_2}_{\text{"path 2"}}\Big] \tag{3.1}$$

wherep is the annual survival probability of an adult tree, f is the number of fruits made by a tree in a year, each containing a single seed, c is the probability that a fruit is eaten by a frugivore and ϵ is the probability that a seed survives the frugivore treatment (e.g. gut passage, seed handling). The quantity in brackets is the average seed survival probability from paths "1" and "2". The probability that a seed from path $i = 1, 2$ survives and develops into an adult tree is g_i. Several hypotheses (Janzen, 1970; Connell, 1971; Howe and Smallwood, 1982b) argue that seeds dispersed by frugivores have higher chances to become adults, i.e. $\epsilon g_2 > g_1$, otherwise frugivory would not have any benefit at all and should be avoided instead.

If frugivory is beneficial because of seed dispersal, then we should expect c to simply evolve towards larger and larger values such that average seed survival and thus fitness increases. This is a necessary condition for frugivory to evolve, but it is not a sufficient condition. Traits that affect attractiveness of the fruit to frugivores, such as their size, nutrient content or pigmentation that makes them more visible, are also expected to be costly in terms of energy and resources that could instead be directed towards making more fruits. In addition, the response from the frugivores towards such traits also depends on the frugivore abundances, physiology and foraging behaviors, thus making plant investments range from highly profitable to unrewarding.

Fitness optimization is further complicated because of the spatial context where dispersal takes place, because this affects the survival probabilities g_i in intricate ways. The chance of a seed becoming an adult depends on several contingencies such as finding and securing space that is free from other plants, the densities and distances from other plants that compete

for resources such as light, and the number of seeds against which a seed can potentially compete during its development. This means that the g_i are variable both in space and in time. In addition, seeds dispersed by frugivores may encounter seeds not dispersed by them, so the independence of paths 1 and 2 suggested by Figure 1 and equation 1 is not the most general scenario, and the probabilities g_1 and g_2 are conditional on the amount of overlap caused by the pattern of frugivore dispersal.

In order to study the evolution of traits that the plants use to profit from animal dispersal services, we therefore constructed an individual based model. In the next two sections we first explain the mechanics of the model in space and time, and then we give the details about the trade-offs relating fruit traits with fruit production and foraging bebavior of the frugivores.

Spatially explicit individual-based model

We model space as a lattice of $n \times n$ sites with absorbing boundaries. Figure 3.1 describes the events that can take place in this spatial context, Table 3.1 lists the variables and parameters involved. A site can be empty, or occupied by at most one tree with a phenotype or trait value z. At the start of year t, a tree survives death with probability p. Trees produce seeds and with a probability m the trait of a seed can mutate, changing its value to $z + \delta$, where δ is a normally distributed mutational step with mean zero and standard deviation σ. The trait value, changed or not, determines the number of fruits f of a tree and the proportion of fruits c that will be eaten by frugivores. The dependence of f and c on the trait z is explained in the next section 2.3 (equations 3.4 and 3.7), and the number of fruits is discrete ($f = 1, 2, 3, \ldots, \phi$). We assume that there is one single seed per fruit.

Seed dispersal takes place in two different ways. By passive dispersal, e.g. by gravity or wind, $(1-c)f$ seeds from a tree disperse evenly to the 8 neighbor sites (Moore neighborhood). By active dispersal, i.e. by frugivores, $cf\epsilon$ seeds disperse across the landscape, where ϵ is the fraction of seeds that survive frugivore treatment (scarification, digestion, etc.). For each tree the frugivores release their seeds at k randomly and independently chosen sites that are free of trees. We assume that $k < cf\epsilon$ because the number of fruits per tree is discrete and much lower than the number of sites ($f \ll n \times n$), so a tree cannot spread all its seeds across the entire landscape because this leads to fractioned seed numbers per site. Seed release patterns can range from clumped (small k values) to scattered (large k values).

At each site a single seed is chosen for further development into a tree. The phenotype of the winning seed is decided by simple lottery, where the probability of a given phenotype winning is equal to its frequency (i.e. proportion of seeds having the phenotype). If the site happens to be already occupied by a tree (this only happens when seeds are dispersed passively), then nothing else happens and the winner is wasted. If the site happens to be empty, then the chances of the winner becoming a tree in year $t+1$ depends on the number of trees $P(= 0, 1, \ldots, 8)$ in the 8 neighboring sites according to the formula $g = g_0 \exp(-\alpha P)$, where g_0 is a density-independent maturation rate and α is a coefficient giving the strenght of density-dependence. This assumption reflects the Janzen-Connell effect (Janzen, 1970; Connell, 1971), whereby a higher density attracts a disproportionate number of host-specific seed predators or pathogens.

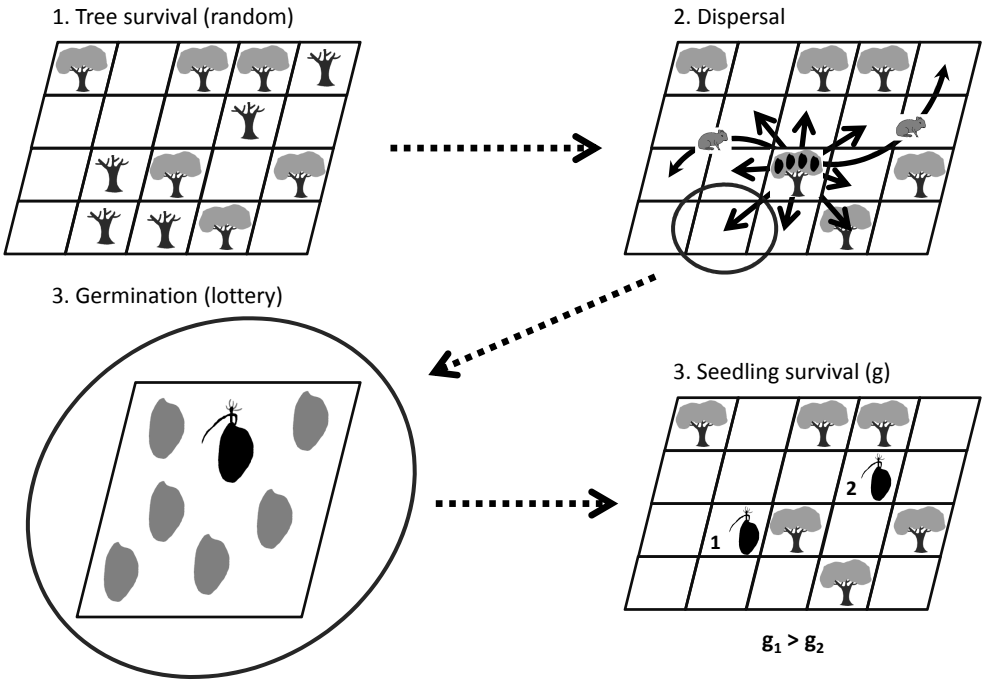


Figure 3.1: Sequence of events in the spatially explicit model of seed dispersal. 1) Adult tree survival: according to annual survival probability p some adult trees survive (trees with leaves) or die (trees without leaves), 2) Passive and animal seed dispersal: each tree disperse their seeds passively to the nearest neighbor cells and actively to different cells in the lattice by frugivores, 3) Lottery competition: seed germination in a patch occurs by lottery competition, i.e. the more abundant phenotype (e.g. small seed phenotype) has a higher probability of germination and 4) Seedling survival: once lottery competition is completed, we evaluate the probability of seedling survival (black seeds) in each patch. Seedling 1 has higher probability of survival than seedling 2 ($g_1 > g_2$) because seedling 1 has fewer surrounding tree neighbors than seedling 2.

Table 3.1: Variables and parameters employed in the simulation model.

Quantity	*Definition*	*Default values*
z	Trait value, e.g. fruit size	$0 < z < 1$
$f(z)$	Number of fruits per tree, discrete variable	$\{1, 2, \ldots, \phi\}$
ϕ	Maximum value of f	100
θ	Cost parameter. Low (high) value means costly (cheap) trait	0.5, 2
$c(z)$	Fraction of fruits eaten by frugivores	$0 < c < 1$
ϵ	Fraction of seeds surviving frugivory	0.9
A	Frugivore abundance	10, 100
ρ	Frugivore choosiness	10
ζ	Frugivore response threshold	0.5
k	Number of sites where the seed of a tree are released	5
p	Adult tree survival probability	0.5
g_0	Maximum of seed to tree survival probability	1
α	Effect of adjacent trees on seed survival	0.01
P	Number of trees is the neighborhood of a site	$\{1, 2, \ldots, 8\}$
m	Probability of mutation on z per tree per year	0.0001
σ	Standard deviation of mutational changes on z	0.025
n	$n \times n$ gives the number of lattice cells	100

The equilibrium of the simulations was checked by estimating whether there were significant differences between replicates of thirty simulations for different parameter combinations and by extending the simulation time to 20000 generations. We found that a simulation time of 10000 generations was always enough to reach an equilibrium.

Fruit production costs and frugivore foraging behavior

Some adaptations are more costly than others, so the number of fruits per tree may depend on the fruit trait under selection in different ways. For example following Smith and Fretwell, 1974, suppose that there is a fixed amount of resources Q per plant set aside for the production of mesocarp, and z is the mass or volume of mesocarp per fruit. Fruits with more mesocarp will be more attractive for animals. Hence, $f(z) \propto Q/z$. Thus, at low values of z an increase in z induces a rapid decrease of f, and we conclude that fruits are very costly. By contrast, suppose that z is the amount of fruit pigment; and more pigment means easy detection and more frugivory. We can argue that pigments are metabolic by-products from the production of compounds that benefit other life-history aspects of the plant (e.g. photosynthetic pigments, secondary metabolites, Cipollini and Levey 1997). In these circumstances the increment in z is not very costly, and the functional form for f may be more like $f(z) \propto a - bz$ where $b \ll a$. Hence, f drops slowly with z, and we conclude that pigmentation is not costly. In general, $f(z)$ must have two properties. The first is that f declines with z:

$$\frac{df}{dz} < 0 \tag{3.2}$$

where the maximum fruit production $f = \phi$ occurs when $z = 0$, i.e. when plants do not invest in attracting frugivores. The second property is that the curvature of $f(z)$ reflects how costly it is to increase the trait, i.e.:

$$\frac{d^2f}{dz^2} \begin{cases} > 0 & \text{high costs} \\ < 0 & \text{low costs} \end{cases} \tag{3.3}$$

For the simulations we need a function $f(z)$ having these properties. A functional relation such as $f(z) \propto Q/z$ satisfies (3.2) and is curved (but only as in $d^2f/dz^2 > 0$); the problem with this function is that it allows the production of infinite numbers of infinitesimally small fruits ($f \to \infty$ as $z \to 0$) and zero production of infinitely large fruits ($f \to 0$ as $z \to \infty$). The functional form $f(z) \propto a - bz$ satisfies (3.2) and keep fruit numbers and trait values bounded, but does not satisfy (3.3) because it lacks curvature. A simple way to model curved trade-offs and bounded fruit production $[0, \phi]$, is by means of the function (see e.g. **?**):

$$f(z) = \phi \left(1 - z^{\theta}\right)^{1/\theta} \tag{3.4}$$

where $f = \phi$ is the maximum fruit production when $z = 0$, and $f = 0$ when z attains an extreme large value that we choose to be 1, without loss of generality. This is represented in Figure 3.2A. When $\theta < 1$ the number of fruits falls rapidly at low values of z, which means high costs ($d^2f/dz^2 > 0$). When $\theta > 1$ the number of fruits falls more slowly at low values of z, which means low costs ($d^2f/dz^2 < 0$). In the simulations f is rounded to the nearest integer.

The probability c that a fruit is eaten by a frugivore is expected to increase with z, but the rate of increase also depends on the abundance of the frugivores as well as on their consumption patterns or behavior. If on the one hand frugivores are very rare, one should expect very low values of $c(z)$ no matter how large the trait, and in fact $c = 0$ if frugivores are absent. If on the other hand frugivores are extremely abundant, fruits have a higher chance to be picked up by at least one frugivore, provided of course that the frugivores like the fruits. This last fact depends in turn on the frugivore response to fruit size, color, nutrients or whatever trait z of interest. If the frugivores are not choosy, $c(z)$ is a saturating function of z, but if the frugivores are choosy then $c(z)$ has a sigmoid shape that becomes more step-like with frugivore choosiness, as shown in Figure 3.2C. Thus $c(z)$ will have the following properties:

$$\frac{dc}{dz} > 0 \tag{3.5}$$

$$\frac{d^2c}{dz^2} \begin{cases} < 0 & \text{: non choosy frugivores} \\ \begin{cases} > 0 & z \text{ small} \\ < 0 & z \text{ large} \end{cases} & \text{: choosy frugivores} \end{cases} \tag{3.6}$$

We propose an analytical form for $c(z)$, following the reasoning behind the Nicholson-Bailey functional response (Nicholson and Bailey, 1935). Given A animals per unit area, with

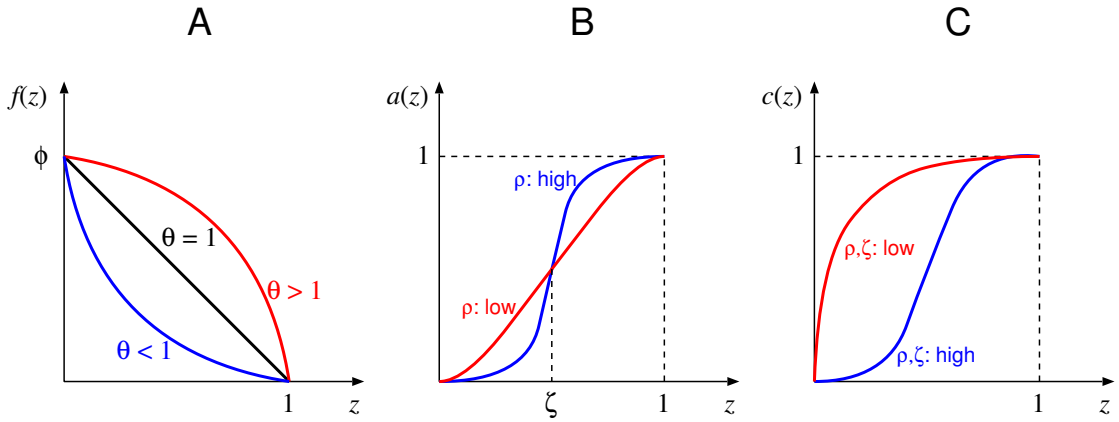


Figure 3.2: (A) Trade-off between fruits per tree f, and the trait that promotes frugivory z (e.g. fruit size or pigmentation). The parameter θ is inversely related with the cost of the trait, e.g. $\theta > 1$ "cheap" (red solid line), $\theta < 1$ "costly" (blue solid line). (B) Fruit consumption rate a as a function of the trait z. The shape parameter ρ measures the frugivore's choosiness: the higher the ρ the steeper the curve and the choosier the frugivores. ζ is the response threshold of the frugivores. (C) Probability c that a fruit is eaten by a frugivore as a function of the trait value z. A high and low ρ curve is shown as a blue and red solid line, respectively.

per-frugivore consumption rate a (i.e. fruits eaten per frugivore, per unit time, per unit area scanned), the probability that a fruit is found and eaten by a frugivore is:

$$c(z) = 1 - e^{-a(z)A} \tag{3.7}$$

The dependence of the consumption rate on the fruit trait is given by a scaled sigmoid function:

$$a(z) = \frac{\frac{1}{1+\exp(-\rho(z-\zeta))} - \frac{1}{1+\exp(\rho\zeta)}}{\frac{1}{1+\exp(-\rho(1-\zeta))} - \frac{1}{1+\exp(\rho\zeta)}} \tag{3.8}$$

where $a(0) = 0$ when the fruit trait is zero and $a(1) = 1$ when the fruit trait takes its maximum viable value $z = 1$ (since $f(1) = 0$ in equation 3.4). Substituting (3.7) in (3.8) we obtain an explicit formulation for $c(z)$. The steepness ρ of the consumption rate determines the choosiness of the frugivores, and the inflection point ζ denotes the frugivore response threshold to the fruit trait. Low values of ζ means that frugivores already start to consume fruits at low values of the fruit trait whereas high values of ζ means that frugivores have high requirements for fruits, i.e. they will start to consume fruits only if they are highly attractive (e.g. color, size). It is important to stress that the response threshold is less important when frugivores are less choosy and more important when they are very choosy. Figure 3.2B, shows the shape of $a(z)$ and Figure 3.2C the final shape of $c(z)$.

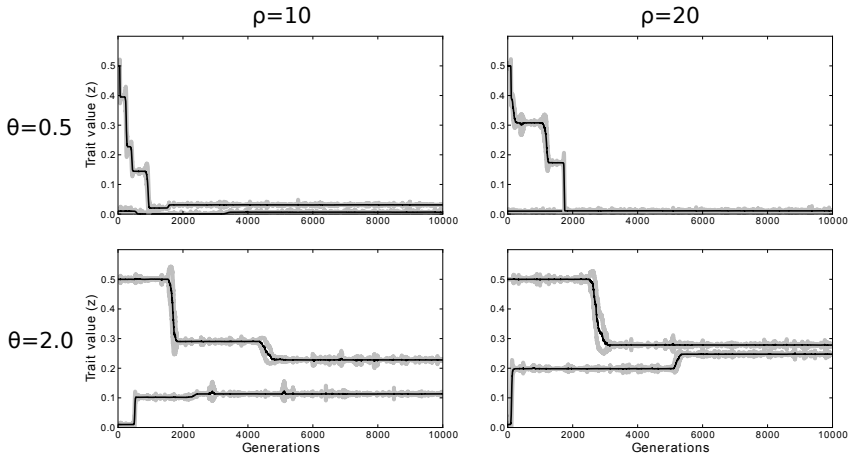


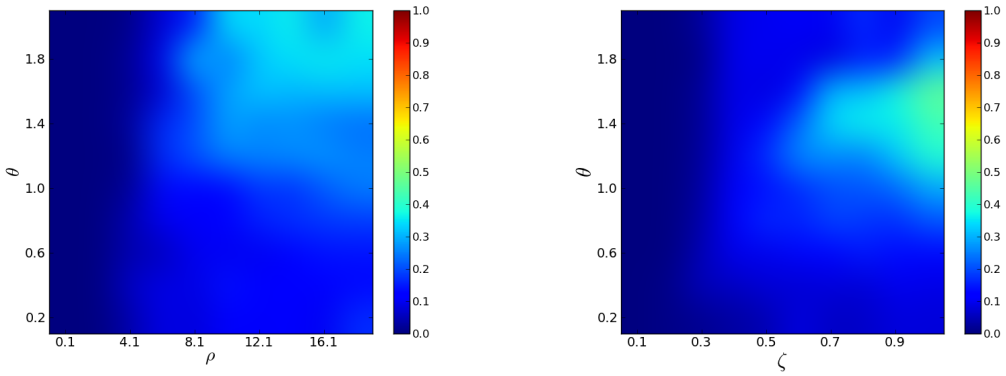
Figure 3.3: Typical trait evolution for two different initial conditions under different values of the cost parameter θ and frugivore choosiness ρ. Black lines correspond to the mean trait in the population and gray lines to the standard distribution. Fruit production costs and choosiness have an important effect on the evolution of frugivory. Costly fruits do not favor the evolution of frugivory, but choosy frugivores can promote it, especially when costs are low. For parameters values used see Table 3.1.

RESULTS

Effect of trait costs and frugivore's foraging behavior

Costs largely determine the extent of trait evolution (Figure 3.3). Under high costs ($\theta < 1$) the trait remains very low or evolves towards very low values, and there is little influence of the frugivore choosiness (ρ) on this outcome. This means that the advantages of attracting the frugivores for dispersal do little to compensate for the associated loss in seed numbers. By contrast, if costs are low or moderate ($\theta > 1$), the trait evolves towards values that are significantly larger (i.e. far from $z = 0$).

Frugivore choosiness appears to be an important driver of evolution when the costs are low or moderate. If the costs are low ($\theta > 1$) the trait tends to evolve to higher values when frugivore choosiness (ρ) increases (Figure 3.3). A similar pattern occurs when the threshold of the consumption rate (ζ) is increased. For lower costs the highest values of the trait occur for large values of the threshold (Figure **??**). A possible explanation for these outcomes is that when costs are not an issue, choosy frugivores and/or frugivores with larger thresholds (large ρ and/or ζ) raise the amount of investment that the plants need in order to profit significantly from their seed dispersal service. By contrast, for non-choosy frugivores and/or frugivores with lower thresholds (small ρ and/or ζ), low values of the trait are already sufficient to cause a large fraction of seeds to be dispersed by frugivores (see Figure 3.2C), so selection for large trait values is rather weak.



(a) (b)

Figure 3.4: Average frugivory trait ($\bar{z}$) values as a function of the cost parameter θ and (a) the frugivore choosiness (ρ), and (b) response threshold. ζ. The average value was taken from ten simulations for each parameter (θ, ρ) and (θ, ζ) combination. In general, decreasing fruit costs and increasing choosiness ($\rho \gg 0$,$\zeta > 0.5$) promote the evolution of frugivory. For parameters values used see Table 3.1.

Effect of frugivore abundance

When the cost of the trait is low ($\theta < 1$) and the frugivores are not choosy (low value of ρ) the trait evolves towards a simple dynamical equilibrium, i.e. there is always a single, global, evolutionary stable strategy (Figure 3.5). In all simulations, we find that the equilibrium value of the trait increases as the number of frugivores decreases. This can be understood as follows: if we consider that when frugivores are rare, passive seed dispersal into neighboring sites predominates over dispersal by frugivores (Fig. 3.5a, top panel), then lottery competition is more intense and seed survival is more difficult due to higher concentration of adult trees around seeds. Under these circumstances, there is a strong selective pressure towards increasing the trait inducing frugivory in order to increase the chances of germination and development (Fig. 3.5a, bottom panel). By contrast, if frugivores are abundant, dispersal by frugivores is already very frequent without requiring much investment by the plant (Fig. 3.5b, top panel). Thus there is weak selection for larger trait values (Fig. 3.5b, bottom panel).

DISCUSSION

Seed dispersal and survival are crucial processes for plant recruitment and population dynamics (Levin et al., 2003). These early developmental stages are critical for plant community dynamics and numerous factors, such as competitive trade-offs (Tilman, 1994), pathogens (Gallery et al., 2010), seed-predators (Avgar et al., 2008) and seed-disperser agents (Schupp et al., 2010) are mediating the evolution of plant dispersal strategies. Several theoretical studies have focused mostly on the ecology of plant recruitment patterns (Nathan and Muller-Landau, 2000) and the evolution of seed-dispersal kernels (Hovestad et al., 2001; Starrfelt and Kokko, 2010), and only few studies have explored the evolution of plant-specific

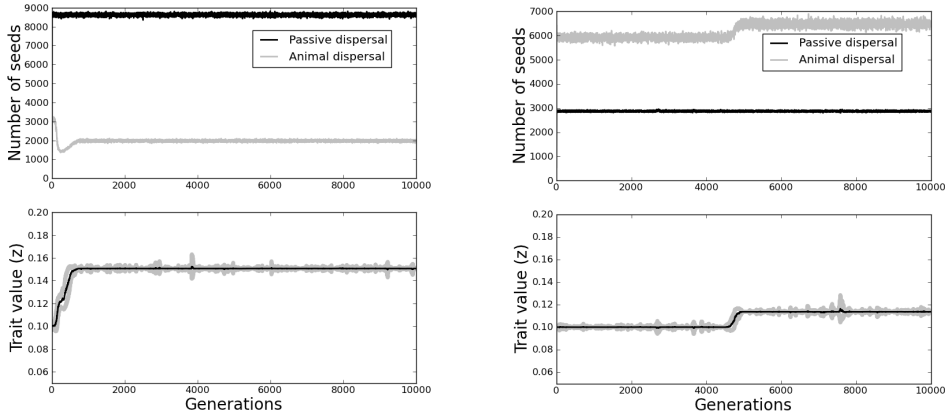
traits in connection with dispersal (Geritz et al., 1999). However, most plants need animal seed-dispersers to survive and reproduce; these animals can even be necessary for the germination of the seeds (Robertson et al., 2005). This is the case for many plants that establish mutualistic interactions with a high diversity of animal frugivores (Traveset et al., 2001). Our study aims to understand how this mutualistic interaction could evolve, in order to explain the high diversity of cryptic fruit traits (e.g. color, aromas) to attract frugivores (Julliot, 1996; Schaefer and Schmidt, 2004; Schaefer et al., 2007, 2008). Our results indicate that the evolution of traits involved in the attraction of frugivores depends on how costly such traits are for the plant and more interestingly, on the abundance and foraging patterns of the frugivores.

Our model has three important advantages compared to previous models. First, it considers different stages in a plant's life cycle, allowing us to account for trade-offs affecting fitness. Second, the plant-animal mutualism is treated as a consumer-resource interaction with benefits for the plants (e.g. dispersal service), enabling us to use principles of consumer resource theory (e.g. functional response, consumer abundance and preferences). And third, it accounts for differences in population regulation encountered by frugivore versus non-frugivore dispersed seeds (e.g. competition for space, seed predation risk, competition with parentals). Foraging decisions form an important feature of the model because frugivores can be highly variable in terms of choosiness and response threshold (Levey, 1987; Schaefer et al., 2003), influencing the extent of dispersal. Our approach is an important step in the direction of "closing the seed dispersal loop" (Wang and Smith, 2002) by merging plant demography and animal foraging behavior. Although we focused on the evolution of fruits, we think that our approach and findings can be applied, with proper modifications, to the evolution of other adaptations required for plant-animal seed dispersal mutualisms, such as the elaiosomes involved in dispersal by ants (Hughes and Westoby, 1990; Giladi, 2006) or the fruit supporting structures in dispersal by bats (Kalko and Condon, 1998).

Drivers of fruit evolution

Fruit production should evolve only if average seed survival increases as a consequence of frugivory, i.e the probability of recruitment from seed to adult is higher in the frugivore recruitment path compared with the non-frugivore path. However, the extent of the evolution is strongly affected by fruit production costs and the availability and foraging behavior of the frugivores (choosiness, response threshold). The picture is further complicated by the fact that the effects of density dependence on survival are heterogeneous in time and space, making the strength of selection for larger fruit production traits very variable.

When the costs associated with traits involved in promoting frugivore dispersal are too high, the traits do not evolve towards significantly larger values in our model, even if frugivore dispersal increases seed survival. However, larger trait values may arise by causes not considered in our model. For example, fruits may have originally evolved as adaptations to protect seeds from predation rather than for dispersal (Mack, 2000), with further evolution driven by the advantages of endozoochory. If the traits are not very costly, then the features (e.g. abundance, choosiness) of the frugivore population will determine the extent of the



(a) A=10 (b) A=100

Figure 3.5: Trait evolution (z) and proportion of passive and frugivore dispersed seeds under two scenarios of frugivore abundance A using cheap fruit costs ($\theta = 2$): a) low abundance ($A = 10$) and b) high abundance ($A = 100$). Decreasing animal abundance promotes the evolution of frugivory. For parameters values used see Table 3.1.

evolution of traits involved in fruit production: If frugivores are very abundant and not very choosy, natural selection favors very small and less colorful fruits, but it favors large and colorful fruits if frugivores are rare and choosy. To understand this outcome, remember that the earliest stages of a plant life cycle, such as seed and seedling, are subject to enormous risks of predation and disease (e.g. granivory, fungi), competition among members of the same cohort (e.g. seedlings competing for nutrients) and competition with other cohorts (e.g. with adults for space and light). Only when a plant attains the adult stage, it becomes relatively free from many of these risks. Frugivore dispersal provides an attractive escape route from these risks. If frugivores become rare and choosy, it pays to invest in attracting them, and natural selection favors larger fruit production traits. If frugivores are very abundant and not choosy, dispersal services would be almost cost-free for plants with small and large fruit traits and there is no selection for larger fruit production traits. A good empirical example of the extent to which plants can adjust to the demands of their dispersers is the plasticity displayed by plants producing watery fruits in summer and nutrient rich ones in winter (Herrera, 1982), both actions would be considered costly, but the changing preferences of the animals force the plants to do so.

An important factor in the evolution is the form of frugivore dispersal, which determines the quality of the service. In our model the frugivore release the seeds only in sites that do not contain trees. We also ran simulations where the frugivores do not discriminate against this condition (not shown here), but in this case the trait always remains at very low values irrespective of the values of the other parameters. This difference in the outcomes occurs because when seeds are released on any site and the landscape fills up (which happens rather quickly), a large number of seeds land on sites occupied by trees, which is lethal.

Exclusive seed release in sites free from trees makes a huge difference in the quality of the dispersal service.

There is still another factor that could determine dispersal quality: the relative degree of clumpiness in the seed release pattern. In our simulations we kept this parameter fixed at 5 sites per adult tree and we have not yet studied the consequences of changing it. Increasing the number of release sites (large k) raises the chances that some seeds recruit far from the vicinity of adult trees, but this also leads to fewer seeds per site which lowers the chances of winning the lottery competition against other phenotypes. It remains to be explored whether this is beneficial or not.

It has been empirically shown that clumped dispersal can severely hinder seed and seedling survival in plants dispersed by monkeys (Russo and Augspurger, 2004), thus creating a potential conflict where frugivore dispersal could be harmful instead of beneficial. The question remains, whether or not clumped dispersal suffices to cause disruptive selection and polymorphism. What has been at least hypothesized in this respect, is that clumped-dispersed plants can develop mechanisms to overcome density-dependence and thus coexist with scatter-dispersed plants (Howe, 1989). Alternatively, plants may induce scattered dispersal by altering gut passage times (which is one of many functions of secondary metabolites (Cipollini and Levey, 1997), such as capsaicin (**?**)).

Beyond simple assumptions

Our model makes several simplifications. We considered scenarios where only mutualists drive the evolution of fruits, but it is important to consider the opposing effects of mutualists and antagonists (i.e. herbivores) . We expect that this promotes trait diversification (Gautier-Hion et al., 1985) and that it has a strong influence on the coevolution between plants and frugivores, as in the case of plant-pollinator interactions (Ferriere et al., 2007). Furthermore, we have not yet considered more specific characteristics of social frugivores, such as monkeys and birds (Russo et al., 2006). They may spend some time travelling between trees compared to the time they spend on foraging in a tree. This will likely cause many frugivores to release seeds closer to a tree in comparison with seeds that disperse passively. In this situation the frugivore may be "cheating", because they obtain the rewards but perform a very poor dispersal service by aggregating the seeds (Russo and Augspurger, 2004).

Fruits are very complex structures that are the product of "phenotypic integration" (Valido et al., 2011), where traits such as color, size and nutrients among others might be signaling for multiple receivers: mutualists and antagonists (Schaefer and Schmidt, 2004). Fruit traits are known to correlate with other plant traits constraining selection by frugivores (Flörchinger et al., 2010). Accordingly, instead of single traits considered one at a time, a linear combination (e.g. principal component) could realistically represent the trait axis along which evolutionary changes happen, and a potential object of study can be the joint evolution of fruit and seed size (Bolmgren and Eriksson, 2010). Mathematical models of seed evolution assume large but costly seeds as adaptations for competition (Geritz et al., 1999), and our model assumes large fruits as dispersal adaptations. It would be interesting to investigate these effects simultaneously.

The coevolution between plant and animal traits was not explored in this model. However, if we also consider evolutionary changes in the animal traits (e.g. choosiness, response threshold) and animal demography, this might promote coevolutionary changes in plant and animal traits and the evolution of dispersal syndromes.

Our model predicts that fruit evolution is determined by frugivore abundance, treated here as a parameter. Realistically, frugivores respond to plant population dynamics, as assumed in most consumer-resource models (e.g. Rosenzweig-MacArthur model). Consumer-resource dynamics will have important ecological and evolutionary consequences, because changes in the composition of frugivore guilds affect plant fitnesses and population viability (Asquith et al., 1999; Wright, 2003; Guimarães et al., 2008). Changes in frugivore's density and/or consumer-resource cycles could potentially generate diversification in fruit traits by, for example, evolving unattractive and highly attractive fruits. This is analogous to the evolution of different levels of resource specialization in consumer-resource interactions (Abrams, 2006).

We predict that the evolution of fruit diversification by frugivory is mainly driven by fruit production costs, but more importantly by frugivore foraging behaviors (i.e.. choosiness, discriminability **?**Kalko and Condon 1998; Schaefer and Schmidt 2004; Schaefer et al. 2007; Flörchinger et al. 2010), and the effects of frugivore seed release patterns on seed survivability and density-dependence (Russo and Augspurger, 2004; Russo et al., 2006). We contend that our approach of considering life-history and consumer-resource theories is essential for the creation of models that seek to explain the evolutionary origin of plant diversification and dispersal syndromes.

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4

PHENOLOGY DRIVES MUTUALISTIC NETWORK STRUCTURE AND DIVERSITY

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Several network properties have been identified as determinants of the stability and complexity of mutualistic networks. However, it is unclear which mechanisms give rise to these network properties. Phenology seems important, because it shapes the topology of mutualistic networks, but its effects on the dynamics of mutualistic networks have scarcely been studied. Here we study these effects with a general dynamical model of mutualistic and competitive interactions where the interaction strength depends on the temporal overlap between species resulting from their phenologies. We find a negative complexity-stability relationship where phenologies maximizing mutualistic interactions and minimizing intra-guild competitive interactions generate speciose, nested and poorly connected networks with moderate asymmetry and low resilience. Moreover, lengthening the season increases diversity and resilience. This highlights the fragility of real mutualistic communities with short seasons (e.g. Arctic environments) to drastic environmental changes.

KEYWORDS: mutualistic networks; phenology; season length; diversity-stability debate; asymmetry; intra-guild competition; nestedness; resilience; connectance

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INTRODUCTION

Mutualism has been suggested to be the mainstay of ecological communities (Bronstein et al., 2006). Mutualistic interactions are ubiquitous in nature and many ecosystems depend on the presence of mutualist services (e.g. pollination) (Bawa, 1980; Janos, 1980). The structure and dynamics of plant-animal mutualistic communities (e.g plant-pollinators, plant-seed dispersers) have been extensively studied (Bascompte and Jordano, 2007b; Vázquez et al., 2009c). The topological description of these networks of interactions has revealed very interesting patterns of how these webs are structured. Mutualistic webs are highly asymmetric (Bascompte et al., 2006; Bascompte and Jordano, 2007b) in terms of degree (i.e number of interactions) and also in terms of interaction strength between mutualist partners (Vázquez and Aizen, 2004; Bascompte et al., 2006; Vázquez et al., 2007). They are also highly nested, nestedness describing how "specialists interact with species that form perfect subsets of the species with which generalists interact" (Bascompte et al., 2003). Nested topologies seem to be temporally invariant (Alarcon et al., 2008; Petanidou et al., 2008) and recent theoretical work indicates that nestedness begets stability and biodiversity (Bastolla et al., 2009). However, it is still not clear what the main mechanisms are that give rise to these topological properties. It has been suggested that there is a combination of niche and neutral processes governing the topology of mutualistic webs (Jordano et al., 2003; Krishna et al., 2008; Vázquez et al., 2009b). Neutral processes driven by random interactions and dispersal are important factors explaining the observed patterns, but niche processes based on biological trait differences are undoubtedly also dominant forces in the evolution and ecology of mutualistic webs (Vázquez et al., 2009b).

Recent studies suggest that these networks are shaped mainly by biological constraints (Vázquez et al., 2009c; Olesen et al., 2010), also called forbidden links. Forbidden links are potential interactions that are not observed due to biological constraints, such as morphological differences, body size or phenological uncoupling (Jordano et al., 2003; Olesen et al., 2010). Phenological uncoupling has been considered one of the most important constraints shaping these webs, explaining around one third of all non-observed interactions (Olesen et al., 2010). Furthermore, Vázquez et al. (2009c) found that species abundance and phenological and spatial overlaps seem to better explain and predict the structure of mutualistic webs than phenotypic traits and phylogenetic relationships do. Abundance and phenology are clearly related because species phenologies (i.e. length of activity during the season) determine: 1) who potentially interacts with whom (and therefore the number of interactions) and 2) relative species abundance in a specific time of the season. Studies of phenology have a long history in the ecological literature (Rathcke and Lacey, 1985), mostly dedicated to describe and quantify the effect of phenological overlaps to explain community composition (Flemming and Partridge, 1984; Feinsinger, 1987) and how species phenologies are affected by abiotic factors (Inouye et al., 2000). More recently, several studies have reported dramatic changes in species phenology by global warming (Penuelas and Filella, 2001; Post and Forchhammer, 2001). Phenological shifts potentially have disastrous consequences for mutualistic community composition (Memmott et al., 2007; Hegland et al., 2009). However, we are still far from making good predictions about the fate of mutualistic

communities under global warming, because we do not yet know the general effects of phenology in the dynamics of mutualistic communities.

Thus, it is crucial to obtain thorough understanding of the role of phenology on mutualistic communities, not only because it is a key factor in the assembly of these webs, but also for the conservation of ecological communities threatened by rapid global changes. Phenology has been poorly studied in theoretical models of mutualistic networks. Theoretical work has focused on studying the effect of phenological shifts in a static network (Memmott et al., 2007) or the network build-up mechanism (Pradal et al., 2009; Kallimanis et al., 2009). Some results suggest that mutualistic network properties can be produced stochastically (Kallimanis et al., 2009). However, there is ample empirical evidence indicating that the assembly of mutualistic webs is not a fundamentally stochastic phenomenon (Jordano et al., 2003; Olesen et al., 2007, 2010). Moreover, some basic questions remain to be answered: to what extent can phenological coupling explain the observed topological patterns? And more importantly how relevant is phenology for the stability of mutualistic webs?

Here, we develop a discrete multispecies population dynamics model based on mutualism and competition that considers the phenology distribution of the species (i.e. their distribution of starting and final dates of activity). We 1) build the network of interactions, 2) determine interaction strengths from the phenological couplings and 3) study their dynamics. We use phenological coupling (i.e. how much temporal overlap exists between two species) as a proxy for species interaction strength, because this simple measure give us a potential estimate of the interaction frequency between species and it allows making inter-community comparisons. Phenological coupling between mutualists give us an indication of how much exchange of resources (e.g nectar, pollen) and/or services (e.g. pollination) potentially occurs between a plant and animal species (Memmott et al., 2007; Miller-Rushing et al., 2010). It can also indirectly give insight into how much competition for resources or services potentially occurs among species of the same guild (e.g. plants or animals) (Feinsinger, 1987; Aizen and Rovere, 2010). Our model allows us to study the effect of phenological distributions on the topology and dynamics of mutualistic webs and, to our knowledge, is the first one to use a biological proxy for interaction strength. In particular, we find that phenology distributions maximizing mutualistic couplings and minimizing competition promote coexistence and generate topological properties observed in real plant-animal mutualistic communities.

MUTUALISTIC COMMUNITY MODEL

Model formulation

Our mutualistic community is composed of two guilds: annual plant and animal species, forming a bipartite network of interactions of n_P ($i = 1, \ldots, n_P$) plants and n_A ($j = 1, \ldots, n_A$) animals. We split the dynamics of the community into: 1) *density-independent dynamics*: in which background mortality takes place and 2) *density-dependent dynamics*: in which reproduction occurs resulting from the phenological overlaps that determine the strength of mutualisms between species of different guilds and competition between species of the same guild

1) *Density-independent dynamics*. A single cohort of P_i (A_j) individuals of plant i (animal j) emerges at day d_i (δ_j). The probability that a plant (animal) survives from one day to the next is S_P (S_A). On day e_i (ϵ_j) all survivors leave seeds (eggs) and die. Thus, the population dynamics of plant and animal cohorts are given by

$$\begin{aligned} P_{id} &= \begin{cases} P_i S_P^{d-d_i} & d_i < d < e_i \\ 0 & \text{otherwise} \end{cases} \\ A_{jd} &= \begin{cases} A_j S_A^{d-\delta_j} & \delta_j < d < \epsilon_j \\ 0 & \text{otherwise} \end{cases} \end{aligned} \tag{4.1}$$

where d is a day of season with season length *(SL)* ($1 \leqslant d \leqslant SL$). This accounts for the fact that abundances are not constant, but decline during the activity season (Pradal et al., 2009).

2) *Density-dependent dynamics*. The relative strenghts of mutualistic and competitive interactions are proportional to the amount of phenological overlap between species. To illustrate this we consider the case of mutualism. If a plant and an animal coincide on the same day, a mutualistic interaction exists and each individual receives one "profit token". Thus, the number of tokens collected by a single plant at day d is the number of animals it meets that day, $\sum_j A_{jd}$. Integrating along the period of activity (*SL*), the profit tokens collected by an individual of plant i will be $\sum_{d=1}^{SL}\sum_{j=1}^{n_A} O_{ijd}A_{jd}$, where O_{ijd} is 1 if i and j coincide on day d, and 0 otherwise. By substituting A_{jd} from (4.1) in this double sum, we can rewrite it as $\sum_{j=1}^{n_A} m_{ij}A_j$, where $m_{ij} = \sum_{d=1}^{SL} O_{ijd}S_A^{d-\delta_j}$ is a mutualistic coefficient, i.e. the annual per capita positive effect of animal j on plant i. In a similar fashion we can define the mutualistic coefficient μ_{ji}, the annual per capita positive effect of plant i on animal j. With respect to intra-guild competition, the coincidence of two plants (animals) in the same day penalizes each species with a "cost token", and the corresponding competition coefficient $x_{ik}(y_{jk})$ (i.e. the annual per capita negative effect of plant (animal) k on plant i (animal j)) is calculated as $x_{ik} = \sum_{d=1}^{SL} O_{ikd}S_P^{d-d_k}$ (see Appendix B for details). Summarizing, the interaction coefficients between two species are the sums of their daily coincidences, weighted by their ever decreasing frequencies due to mortality. Note that in the case of $i = k$ ($j = k$), x_{ii} (y_{jj}) is an intra-specific competition coefficient. In contrast with inter-specific competition coefficients that can be zero if two species never coincide, intra-specific coefficients are never zero, because a species always coincides temporally with itself. Furthermore, the phenophase of a species cannot be shorter than its overlap with other species, which implies that intra-specific competition coefficients can never be smaller than inter-specific competition coefficients.

The number of seeds (eggs) produced by a plant (animal) depends on the balance of positive (mutualisms) versus negative (competition) effects experienced during the season. According to equation (4.1) the number of reproducing plants (animals) of species i (j) at the ending day d_i (δ_j) is $P_i S_P^{e_i-d_i}$ ($A_j S_A^{\epsilon_j-\delta_j}$). We model reproduction as a multispecies version of the Ricker map, such that, when combined with equation (4.1), the plant and animal population sizes at year $t+1$ are related to the sizes at year t as:

$$
\begin{aligned}
P_i' &= P_i S_P^{e_i - d_i} r \exp\left(\frac{\sum_j m_{ij} A_j}{h_P + \sum_j m_{ij} A_j} - b \sum_k x_{ik} P_k\right) \\
A_j' &= A_j S_A^{\epsilon_j - \delta_j} \rho \exp\left(\frac{\sum_i \mu_{ji} P_i}{h_A + \sum_i \mu_{ji} P_i} - \beta \sum_k y_{jk} A_k\right)
\end{aligned} \tag{4.2}
$$

where r (ρ) is a growth rate scaling factor. The per capita reproduction rates are increasing but saturating functions of the strength of the mutualistic interactions (this ensures that population dynamics are bounded (Vandermeer and Boucher, 1978)); h_P (h_A) is a half-saturation constant. The negative effects of competition are simply additive (Bastolla et al., 2009). Longer phenophases are expected to produce larger overlaps and thus higher mutualistic (m_{ij}, μ_{ji}) and intra-guild competitive (x_{ik}, y_{jk}) effects. The relative strength of competition against mutualism depends on the scaling factor b (β).

In this model mutualism can be *obligate* or *facultative*. Obligate mutualists have baseline growth rates smaller than one ($rS^{e_i - d_i} < 1$, $\rho S^{\epsilon_j - \delta_j} < 1$, $S < 1$), and in the absence of mutualistic interactions they always go extinct (even when competitors are absent). Facultative mutualists have baseline growth rates larger than one ($rS^{e_i - d_i} > 1$, $\rho S^{\epsilon_j - \delta_j} > 1$, $S < 1$). Facultative species can grow in isolation (i.e. in the absence of mutualism and competition), but in the presence of other species their fate depends on the balance between mutualism and competition. In the absence of mutualism, facultative species may coexist or not with other species or the same guild; this depends on the matrix of competition coefficients and the other model parameters (Strobeck, 1973). Facultative species may need mutualistic partners to avoid competitive exclusion or to increase their dominance. The proportion of obligate and facultative species in the model depends on the season length. This is because a shorter season entails shorter phenophases whereas shorter phenophases are associated with higher average baseline growth rates ($rS^{e_i - d_i} > 1$, $\rho S^{\epsilon_j - \delta_j} > 1$, $S < 1$) and higher average baseline growth rates allow a higher proportion of facultative to obligate mutualists. Most of the model analysis was done with facultative species (see sub-section: "Phenology distributions and parameter settings" for details).

MODEL ANALYSIS

We employed numerical simulations to study the effect of phenology on network topology, community stability and biodiversity.

Phenology distributions and parameter settings

Previous work indicates that the distribution of phenologies or phenophases in mutualistic communities is right-skewed (Rathcke and Lacey, 1985; Kallimanis et al., 2009), and particularly that the phenology of flowering plants is log-normally distributed (Bawa, 1980; Kallimanis et al., 2009). We tested various distributions and decided to use a log-normal distribution for the generation of phenology distributions (see Appendix B for details): the starting dates (d_i, δ_j) are log-normally distributed variables with mean μ_d and variance σ_d^2

of the corresponding normal distribution of the logarithm of starting dates ($\log(d)$) and the phenology lengths (p_i, p_j) are log-normally distributed variables with mean μ_p and variance σ_p^2 of the normal distribution of the logarithm of phenology lengths ($\log(p)$). The ending dates (e_i, e_j) are calculated summing the values of the starting date (d_i) and phenology length (p_i), such that: $e_i = d_i + p_i$. The mean starting date was set to $\mu_d = 1$ and the mean phenology length is $\mu_p = 1$. The different season lenghts $SL = 6, 18, 30, 300$ set the maximum value of phenology length (p_i) possible for any species. The variances were varied in the range $[0, 6]$. As we explained before, in our model we can have facultative and/or obligatory mutualistic species depending on the season length and parameter settings chosen. For example, using the parameter values: $r = \rho = 1.5$, $b = \beta = 1.5$, $h_A = h_P = 1$ and $S_P = S_A = 0.99$, we have 100% facultative species for $SL \leqslant 40$ days and facultative as well as obligatory species for $SL > 40$ days. We did most of the analysis with 100% facultative mutualists ($SL = 18$).

We set $r = \rho = 1.5$, $b = \beta = 1.5$, $h_A = h_P = 1$ and $S_P = S_A = 0.99$ based on our sensitivity analysis to assure persistence and stability. We simulated the community dynamics 120 times for each combination of variances of starting dates and phenology lengths ($\sigma^2_{d,i}, \sigma^2_{p,j}$). The initial community diversity consisted of 60 plant and 60 animal species densities randomly chosen from an uniform distribution. We checked for different community diversities $n_A, n_P = 50, 60, 70, 100, 120$ and different ratios of plants to animals $1:1, 1:2, 1:3$, but we did not find qualitatively important differences. The running time of the simulations was 3500 years, which is more than enough to ensure the convergence to an attractor. A species was removed and considered extinct if its density fell below 10^{-8}. The model was fully implemented in MATLAB 7.6 (Mathworks Software, 2008).

Stability, resilience and community diversity

We determined community stability by means of the variance of all population dynamics over the last 500 time steps of the simulation. We declared a community as stable if this variance was less than 10^{-6}. For stable communities we determined the leading eigenvalue λ_1 of the Jacobian matrix of the dynamical system (4.2), and computed resilience as the return rate to a stable equilibrium after a small perturbation:$-\log(\lambda_1)$ (DeAngelis, 1980). Because communities also change due to extinctions, we recorded initial and final values for community statistics (size, diversity) and network properties.

Network properties: nestedness, connectance and asymmetry

Nestedness in mutualistic networks is defined as the degree to which specialists interact with proper subsets of the species interacting with generalists (Bascompte et al., 2003). We calculated nestedness in two ways: 1) the temperature of the interaction matrix (Atmar and Patterson, 1993) and 2) the NODF algorithm (Almeida-Neto et al., 2008). We then calculated relative nestedness (N^*) as a measure of how nested a network is, compared to the mean expected value from a null model ($\overline{N}_r$): $N^* = (N - \overline{N}_r)/\overline{N}_r$ (see Appendix B for details). We only reported nestedness values using the NODF algorithm because both metrics gave very similar results and NODF is less prone to type I errors (Almeida-Neto

et al., 2008). Mutualistic and competitive connectance was also calculated for each network. Connectance is a measure of the proportion of realized interactions among all possible interactions in a network. Mutualistic connectance occurs between animals and plants and competitive connectance only between plants (animals). The asymmetry of the interaction strength between plants and animals was calculated using relative dependence values as in Bascompte et al. (2006) (see appendix B for details).

Statistical analyses

We performed statistical analyses in R 2.10.1 (R Development Core Team, 2010) to test the effect of network structure (nestedness, asymmetry and connectance) on the final community diversity and resilience. We used generalized linear models (GLM) because our data had nonnormally distributed errors. We used Gamma GLM models with identity-link (Bolker, 2008) to test the effect of network structure on community resilience and Poisson regressions to test the effect on final community diversity. We assessed the significance of the most adequate model by an analysis of deviance (Likelihood ratio tests) on a nested sequence of models using a forward elimination process, going from a full model with two-way interactions to a minimal adequate model; p values were used to evaluate the elimination process. Furthermore, we studied the effect of season length on network structure, diversity and resilience of final communities using Kruskal–Wallis one-way analysis of variance.

RESULTS

Community diversity

Each sampled phenology distribution generates a specific network structure of interactions, depending only on the variances of starting dates (σ_d^2) and phenology lengths (σ_p^2) and on the season length (*SL*). Network properties change drastically from their initial values to various equilibrium values. Species extinctions often occur during the dynamical process, creating different community diversities depending on the variation of starting and ending dates. Highest community diversity is reached when both variances were low and equal ($\sigma_d^2 = \sigma_p^2 < 3$) (*LEV*) (see figure 4.1b). Furthermore, the variance of starting dates (σ_d^2) seems to be more important for increasing coexistence of species than the variance of phenology lengths (σ_p^2). This is because the variance of starting dates determines the spread of species phenologies across the season. Summarizing, smaller variances in starting dates and phenology lengths lead to higher diversity.

Mutualistic and competitive connectance

The connectances of mutualistic and competitive interactions are very similar (figures 4.1e,4.1f and B.2a, B.2b) and hence highly correlated for initial ($R^2 = 0.678, p < 0.001$) and final values ($R^2 = 0.952, p < 0.001$), regardless of the phenology distribution variances. This dependence is due to the symmetry between plant and animal phenologies (figures B.8a and B.8b); hence changes in the connectance of mutualistic interactions are not independent

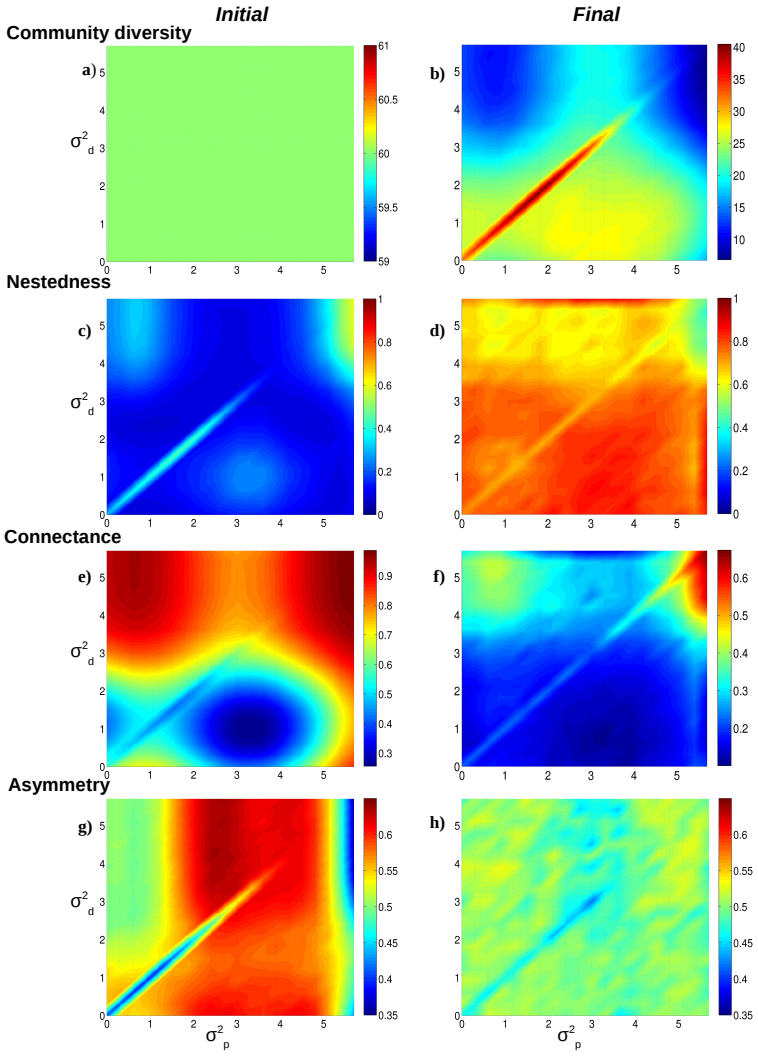
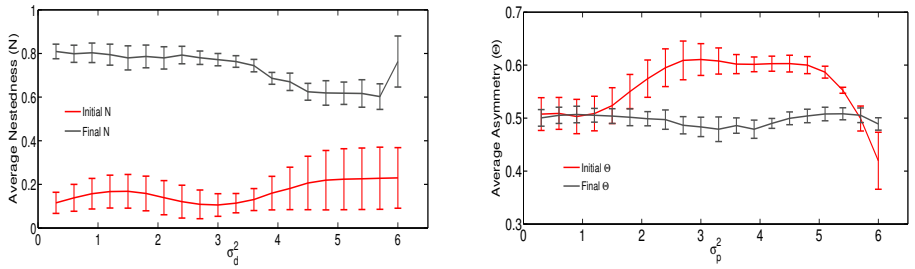


Figure 4.1: Network structure changes. The left column (panels a, c, e, g) represents initial values and the right column (panels b, d, f, h) represents final values of different network properties and community diversity. All network properties (nestedness, connectance and asymmetry) have profound changes. Results are averaged over 150 simulations for each phenology distribution combination (σ_d^2, σ_p^2). The season length is $SL = 18$. Initial community diversity was $P = 60$ and $A = 60$, for plants and animals, respectively. Demographic parameter values used: $r_i, \rho_j = 1.5$, $b_i, \beta_j = 1.5$ and $S_A, S_P = 0.99$. Nestedness values were all significantly different from null model estimates ($p < 0.05$)



(a) (b)

Figure 4.2: Changes in a) nestedness and b) asymmetry with variance in starting date and phenology length. Nestedness shows greater variation due to changes in the variance of starting dates and asymmetry shows higher variation due to changes in the variance of final dates. Red and grey solid lines indicate initial and final values, respectively. The size of the bars represents the standard error values for each average value (SL = 18).

of the connectance of competitive interactions. For example, a fully coupled mutualistic network in our model implies a fully coupled web of competitors and vice versa. Because of this high correlation, we report only the mutualistic connectance.

Network structure changes

Network dynamics changes several structural properties of the initial network configurations. Nestedness increases for all communities irrespective of the variance of their phenology distribution (figure 4.2a). *LEV* and high variance communities have the highest initial nestedness (figure 4.1a) and nestedness increases only slightly for these communities. Connectance (mutualistic and competitive) decreases in all communities irrespective of the variance of their phenology distribution (figure 4.1 f). This indicates that mostly species that are highly connected are eliminated during the dynamics. Even though nestedness increases and connectance decreases in all simulated communities, the amount of change depends on the variance of starting dates (σ_d^2) (figure 4.1).

The average asymmetry per species between plants and animals is initially different across simulated communities but very similar in final stable communities (figure 4.2b) and they reach an intermediate value of asymmetry ($\overline{\Theta} \approx 0.5$) (figure 4.1h). This indicates that stable communities have many phenological couplings between species of similar phenology lengths and very few highly asymmetrical couplings between species of different phenology lengths (figure 4.6). *LEV* communities ($\sigma_d^2 = \sigma_p^2 < 3$) have the lowest average asymmetry ($\overline{\Theta} \approx 0.4$) (figure 4.1g) because they have phenology distributions well spread across the season and phenological couplings between mutualists are very symmetrical. High equal variance communities ($\sigma_d^2 = \sigma_p^2 > 5$) also have low asymmetry because they have many couplings between species of similar phenology lengths (figure B.1) as well.

High asymmetry is expected when $\sigma_p^2 \neq \sigma_d^2$; i.e. when there is a large number of phenological couplings between species of different phenology lengths (figure 4.1g).

Network structure and survival rate

We assume in our model that species abundances decrease proportionally to their phenology length with a daily survival rate of $S = 0.99$. We checked the effect of this assumption through numerical simulations by setting $S = 1.0$ (see Appendix B for details). Our results shown in appendix B indicate that our assumption ($S = 0.99$) does not change the network structure qualitatively. The most conspicuous quantitative change is that the final connectance increases much more ($\bar{C}_f = 0.74 \pm 0.14$) with $S = 1.0$ than with $S = 0.99$. Therefore, if we assume the unrealistic scenario that species survive throughout the season (i.e. abundances are constant), we increase the species probability to have more interactions and this is particularly true for species with longer phenologies.

Relationships between network properties

Our results show that initial communities with high nestedness and lower connectances are those associated with larger community diversities and fewer structural changes. Main network topological properties are associated with increases and decresases of community diversity. Decreasing connectance ($GLM, F_{1,47998} = 296702.19, p < 0.01$) (figure 4.3b) and increasing nestedness ($GLM, F_{1,47998} = 278844.87, p < 0.01$) are associated with higher diversity. But increasing interaction strength is negatively associated with community diversity ($GLM, F_{1,47998} = 218431, p < 0.01$). We also find that community diversity seems to reach an optimum at moderate asymmetry ($GLM, F_{1,47998} = 54528.61, p < 0.01$). This is because communities with average values of diversity (≈ 25), typically of phenology distributions with $\sigma_p^2 \neq \sigma_d^2$, have higher asymmetry. Thus, some level of asymmetry is needed to obtain higher coexistence of species; i.e. the presence of few species with long phenologies ("generalists") is associated with higher coexistence. These "generalist" species allow some species with short phenologies to survive. But, having very high asymmetry will increase competition.

In summary, highly diverse communities are associated with high nestedness, low connectance and moderate asymmetric interactions.

Phenology lengths distributions

The distribution of phenology lengths become highly heterogeneous, irrespective of community diversity. Communities with many species with short phenologies and few species with long phenologies seem to be more stable (figure 4.5).

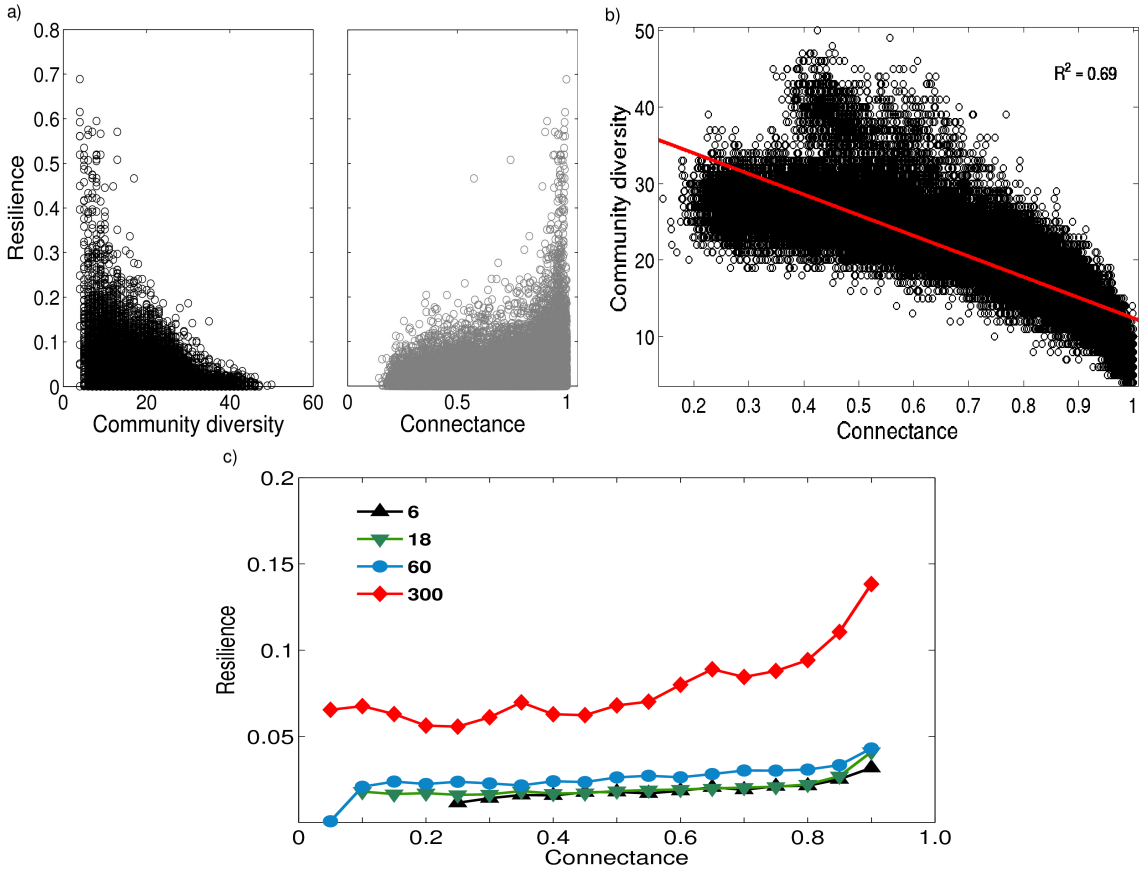
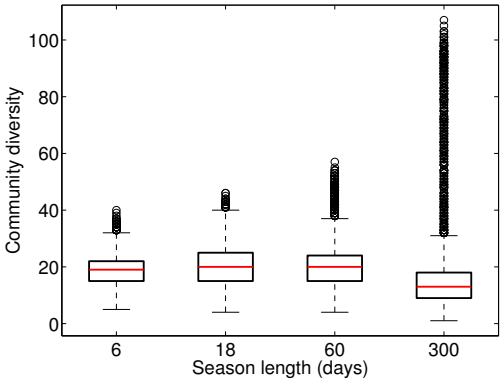
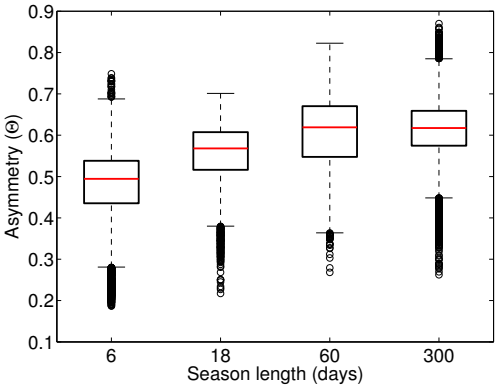


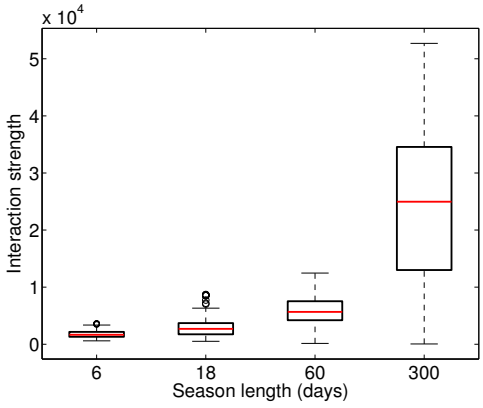
Figure 4.3: Relationships between connectance, resilience and community diversity. a) Effects of community diversity and connectance on resilience. Left panel shows the resilience for different community diversities (black open circles) and the right panel shows the resilience for different initial connectance values (grey open circles). All phenology distributions were sampled from $\sigma_d^2, \sigma_p^2 \rightarrow [0, 6]$ and SL = 18 days. b) Community diversity as a function of connectance. There is a negative correlation between diversity and connectance. Red solid line is a linear regression fit ($R^2 = 0.69$). Each open circle represents a simulated community. c) Effect of season length on connectance and resilience. Resilience strongly increases for long *SL*. Each symbol represents an average value of resilience for a value of connectance (C) and *SL*. Data from 46,500 simulations and SL = 6, 18, 60, 300.



(a)



(b)



(c)

Figure 4.4: Effects of season length (*SL*) on community diversity, asymmetry and interaction strength. a) Season length and community diversity. Maximum community diversity increases with *SL*. b) Season length and asymmetry c) Mutualistic interaction strength and their variation strongly increase for long *SL*. The black box has lines at the lower quartile, median, and upper quartile values. Red solid lines represent median values and black open circles are outliers. Data from 46,500 network simulations from different phenology distributions and four season lengths (SL $= 6, 18, 60, 300$).

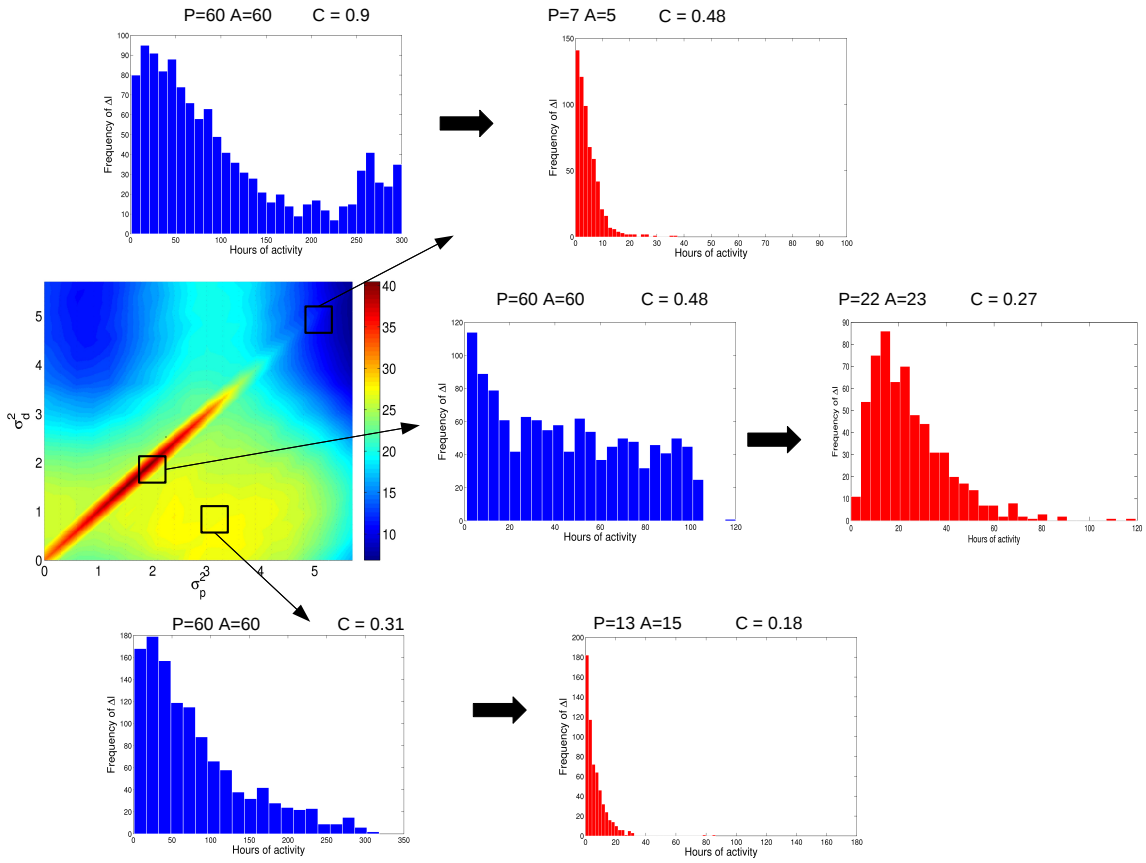


Figure 4.5: Change in phenophase distribution for different community diversities. Highly right-skewed phenology distributions are produced in all final communities independent of their initial phenology distribution. Blue and red bars represent initial and final phenophase distributions for the whole community, respectively. Thin black arrows indicates phenophase distributions for different final community diversities (SL = 18).

Stability and resilience

97% of the simulations achieve a stable community equilibrium at the end of the simulations. Phenology distributions with high variances ($\sigma_d^2, \sigma_p^2 > 5$) generate highly resilient communities and *LEV* phenology distributions generate communities with low resilience (see figure B.3). This suggests that species poor communities have higher resilience than more diverse communities. Indeed, resilience is inversely related to community diversity (figure 4.3a). Connectance is our best network structure predictor for community resilience (GLM, $F_{1,46478} = 1742.4, p < 0.01$). Nestedness does not show a clear relationship with resilience. However, highly nested communities were more resilient than non-nested ones (figure B.4a).

Season length

Season length (*SL*) is a crucial factor for the stability and diversity of mutualistic communities in our model. Increases in *SL* produce more stable networks (figure B.6) and higher resilience. Mean community diversity does not change with *SL*; but diversity variation and the maximum diversity reached increase (figure 4.4a). Maximum diversity increases with *SL* because of higher temporary availability of niches for networks that promote diversity, i.e. networks that maximize mutualistic couplings and minimize competitive couplings. Furthermore, community diversity variation increases with *SL* because of more variation in network configurations, in terms of connectance and interaction strength. However, nestedness is not increased by increases in *SL* (figure B.5), which suggests that nestedness is only affected by the variances (σ_d^2, σ_p^2) of the phenology distributions.

Resilience increases with *SL* in all communities, but it increases more for highly connected communities ($C > 0.5$) than for poorly connected ones ($C < 0.5$) (figure 4.3c). Furthermore, the mean connectance of highly diverse communities (i.e. *LEV* communities) increases more in communities of longer *SL* ($\overline{C}_{y=300} = 0.631 \pm 0.04$) than in communities of shorter *SL* ($C_{y=18} = 0.461 \pm 0.06$). Thus, an increase in *SL* can generate more diverse and more resilient communities, especially when they are highly connected. Average asymmetry ($\chi^2_{3,139197} = 12155, p < 0.01$) and interaction strength ($\chi^2_{3,139197} = 53858, p < 0.01$) increases with *SL* (figures 4.4b and B.7).

Summarizing, communities living in long *SL* environments are more resilient and asymmetric than communities living in short *SL* environments. The increase in resilience with *SL* is largest for highly connected communities.

DISCUSSION

Several theoretical studies on mutualistic networks have made major progress studying the conditions for stability and coexistence of species (Bascompte et al., 2006; Okuyama and Holland, 2008; Bastolla et al., 2009; Thébault and Fontaine, 2010). The observations of topological properties of mutualistic webs have raised new questions about the mechanisms behind these properties (Vázquez et al., 2009b). Here, we have shown that using a simple biological constraint on the assembly of mutualistic communities already provides more

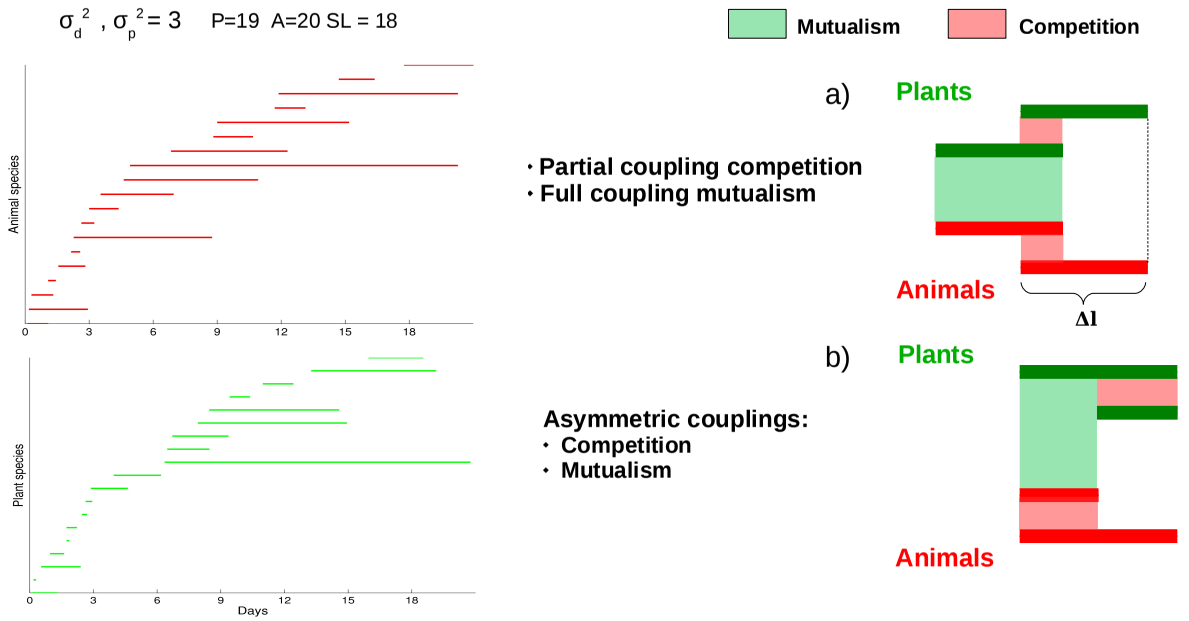


Figure 4.6: Phenological couplings on *LEV* communities. The left column shows the final distribution of phenologies of *LEV* communities composed of plant (green solid lines) and animal (red solid lines) species. The length of these lines represents the phenology length (p) located in a temporal scale across the season. The right column shows the different types of mutualistic (light green areas) and competitive (light red areas) phenological couplings observed in *LEV* communities. a) *LEV* communities have a large number of fully mutualistic (highly symmetrical) and partial competitive couplings (top panel). b) Non-*LEV* communities ($\sigma_d^2 \neq \sigma_p^2$) tend to have more asymmetrical couplings (bottom panel). Season length used is $SL = 18$.

insight into the natural emergence of network topological properties. Phenology, without invoking other biological constraints, can largely explain the main topological properties observed in real plant-animal mutualistic webs. The distribution of phenologies in our model greatly influences the stability and coexistence of species. Right-skewed phenology distributions with starting and final dates well spread across the season (i.e. *LEV* communities) maximize phenological couplings among mutualists and minimize intra-guild competition, thus maximizing diversity. These diverse networks are highly nested and poorly connected similar to real plant-animal mutualistic networks (Jordano et al., 2003; Bascompte et al., 2003), but they have low resilience. The diversity and stability of mutualistic webs are also highly affected by the length of the season, which emphasizes the importance of abiotic factors in the assembly of these communities.

Highly diverse communities are highly nested and moreover, over time nestedness increases in all communities, regardless of their phenology distribution. This is in agreement with Bastolla et al. (2009), who showed that nested structures minimize interspecific competition promoting stability and species coexistence on mutualistic webs. However, in contrast to Bastolla et al. (2009) we do not find that fully connected networks have higher diversity. On the contrary, fully connected networks (i.e. with many of phenological couplings) in our model generate lower diversity because of high intra-guild competition. This agrees with empirical evidence that minimizing phenological overlaps increases the reproductive output of flowering plants (Aizen and Rovere, 2010). We argue that phenology is an important mechamism for the emergence of nested structures. However, neutral processes may also give rise to these patterns (Krishna et al., 2008). Thus, different processes, niche-based and neutral are acting simultaneously on the assembly of mutualistic webs (Krishna et al., 2008).

We found a negative relationship between complexity and stability. The longstanding debate about the diversity-stability relationship started by arguing that complexity (i.e. number of species and/or interactions) promotes ecosystem stability (MacArthur, 1955). This was later questioned by May (1973), who demonstrated that randomly wired food-webs are more unstable when diversity and/or connectance are high [1]. This and subsequent studies stressed the importance of community structure for diversity-stability relationships (Yodzis, 1981). In the mutualistic network literature, a positive diversity-stability relationship has been reported for two different models (Okuyama and Holland, 2008; Thébault and Fontaine, 2010). In contrast, our results indicate a negative relationship: highly diverse communities have low resilience and low connectance. Furthermore, in all communities connectance decreases during the dynamical process due to intra-guild competition. This indicates that extinction of highly connected species occurrs frequently in all communities and more often in highly connected communities. On the one hand, initially highly connected communities suffer more extinctions (i.e. high phenological couplings), but their connectance remain relatively high and they are more resilient. On the other hand, poorly connected communities (e.g. LEV communities) suffer less extinction by competition hence increasing diversity, but producing communities with low resilience.

Our model indicates that connectance is a key factor controlling resilience. Increases in connectance promote stability, but also increase competition. Connectance can be increased

1 (With a fixed community diversity and average weak interaction strength. The condition for stability found by May (1973) is: $s\sqrt{mC} < 1$, s: average interaction strength, m: community diversity and C: connectance

by simultaneously increasing mutualistic and competitive phenological couplings. However, to minimize competition, connectance has to decrease. This also means that mutualistic couplings decrease, but *LEV* communities only decrease marginally. Thus, high coexistence of species is only possible if communities have low connectance in order to minimize intra-guild competition at the cost of having lower resilience. The simultaneous increase of diversity and decrease of connectance drives the communities towards an instability boundary, supporting the idea that ecosystems evolve toward a state of self-organized instability (Solé et al., 2002a). As stated above, previous studies on mutualistic networks reporting a positive effect of connectance on stability, have found a positive diversity-stability relationship (Okuyama and Holland, 2008; Thébault and Fontaine, 2010), contrary to our results. However, these studies did not consider inter-specific competition in the dynamics and the importance of competition has been shown to be crucial for understanding the emergence of structural properties (Bastolla et al., 2009). Furthermore, we find that mutualism can be very damaging for species coexistence when competition is strong (see Appendix B for results analyzing the dynamics with mutualism and competition separately). The balance between positive and negative interactions driven by phenological couplings is what finally determines the stability and coexistence of species in our model. Our results are in agreement with other studies indicating that simultaneous increases of diversity and connectance generate more instability (May, 1973; Gross et al., 2009). However, there are other mechanisms and constraints in the assembly of mutualistic webs, such as phenotypic complementarity (Rezende et al., 2007a), which might contribute to the stability-diversity relationship. Mutualistic communities are obviously much more complex than we modelled. For example, mutualistic communities might also have antagonistic interactions (e.g. herbivory, parasitism) (Bronstein et al., 2003a) and including these interactions in plant-animal mutualistic dynamics can bring new insights to the complexity-stability relationship.

There is an inherent asymmetry of interaction strength between plants and animals (Vázquez and Aizen, 2004; Bascompte et al., 2006). However, stable communities are not characterized by high levels of asymmetry. They show intermediate levels with little variation among communities. We conjecture that communities are composed of different levels of asymmetries. Highly diverse communities, in particular, show low levels of asymmetry compared to other communities, because phenological distributions maximizing mutualistic couplings decrease their level of asymmetry by forming highly overlapping phenologies. Our highly diverse communities appear to be less asymmetric than empirical ones (Bascompte et al., 2006; Vázquez et al., 2007), but observed measures may be overestimating the true asymmetry (Bosch et al., 2009) or highly diverse communities may not be resilient enough, so they are not observed in nature. Even though highly diverse communities tend to have more symmetric interactions, we find that asymmetry is an important factor for species coexistence and stability. Moderate, but not high levels of asymmetry are necessary for species coexistence and stability.

The relationship between asymmetry and resilience is masked by the effect of season length (*SL*). For short *SL*, we were unable to detect an effect of asymmetry on the resilience of communities. However, the positive effect of asymmetry on community resilience and diversity becomes clearer for longer *SL*. Short *SL* communities cannot produce a high propor-

tion of asymmetrical interactions. An increase in *SL* boosts the proportion of communities with moderate asymmetry levels promoting coexistence and resilience.

There are conflicting studies with respect to the effect of asymmetry on the diversity and stability of mutualistic webs. The debate has centered around the questions whether weak asymmetric (Bascompte et al., 2006) or strong symmetric (Okuyama and Holland, 2008) interaction strengths increase stability and diversity. The main difference between models is the type of functional response used to decribe the mutualistic interaction. We used a saturating functional response because it better describes a consumer-resource interaction and avoids population overgrowth by large positive feedbacks (Vandermeer and Boucher, 1978).

Season length clearly affects diversity and stability of mutualistic communities in our model. Empirical evidence shows that it is a limiting factor for the variation of phenological coupling (Olesen et al., 2010). The rise in resilience and diversity for increases in season length seems to be generated by moderate asymmetry levels, high nestedness and connectance. Naturally, with increases in diversity, the number of interactions increase (Solé et al., 2002a) augmenting resilience. Thus, a small window of interactions has a detrimental effect on the stability and diversity of communities, suggesting that communities living in short season lengths environments are vulnerable to perturbations or drastic changes driven by climatic conditions. Evidence is accumulating that high latitude communities are under severe threat of global warming (Post et al., 2009). Recent reports indicate that phenologies are changing quickly, but differentially depending on the species, creating phenological uncouplings at different trophic levels (Post et al., 2008b). We cannot predict how exactly the structure of short season length communities will be affected, but we do argue that any high perturbation could produce a disruption in the network structure and eventually a cascade extinction effect, as shown previously (Memmott et al., 2007).

We assume facultative mutualistic interactions, following previous models (Bastolla et al., 2009; Okuyama and Holland, 2008). However, we want to stress that regardless of the type of mutualism (obligatory or facultative), mutualism is important for the diversity and stability of the community. Under the always present competitive pressure in a guild, species without mutualistic interactions are at a serious disadvantage compared to those that are involved in mutualistic interactions. Thus, mutualism is crucial for a species persistence.

Using phenological coupling as a proxy for interaction strength we have provided insight into the emergence of network structural properties in mutualistic communities. Nevertheless, considering coevolutionary processes of different biological constraints in the dynamics of community assembly can give us even more insight. There is a phylogenetic signal in the way species interact (Rezende et al., 2007a) and mutualistic webs are highly modular (Olesen et al., 2007). Thus, future research should consider the inclusion of other biological constraints and their coevolution.

We have shown that one single biological mechanism, phenology, produces the emergence of several observed patterns in mutualistic communities. The emergent patterns are not caused by a purely stochastic phenomenon, as suggested previously (Kallimanis et al., 2009), but interdependent network properties emerged naturally. Other ecological networks (e.g. food-webs) are also highly affected by the timing of life history events (Post et al., 2008b). Certainly, phenological uncouplings are driving drastic changes in high latitude

communities and ecosystems (Post et al., 2009). Thus, we believe that the role of phenology and spatio-temporal variability are pertinent to the study of community assembly, especially in communities that are highly threatened by global warming.

ACKNOWLEDGEMENTS

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APPENDIX A

Dynamical model

Binary overlap matrices and interaction effects

We assume that two individuals of different guilds interact mutualistically if they coincide on the same day, giving a "profit token" to both partners. Thus, the number of tokens collected by a single plant on day d is equal to the number of animals on that day $\sum_j A_{jd}$; and the number of tokens collected by a single animal on day d is equal to the number of plants on that day $\sum_i P_{id}$. Integrating along the season, the profit collected by an individual of plant i and animal j is respectively

$$\begin{aligned} F_i &= \sum_{d=1}^{SL} \sum_{j=1}^{n_A} O_{ijd} A_{jd} \\ G_j &= \sum_{d=1}^{SL} \sum_{i=1}^{n_P} \Omega_{jid} P_{id} \end{aligned} \tag{A.3}$$

where O_{ijd} and Ω_{jid} are the binary overlap matrices of the mutualistic interaction on day d. If plant i and animal j are present at day d then $O_{ijd} = \Omega_{jid} = 1$, but if any of the two or both are absent then $O_{ijd} = \Omega_{jid} = 0$. The number of individuals of each species decreases with time according to equation 1 in the main text. By substituting equation (1) in equation (A.3) and commuting the summations we arive at

$$F_i = \sum_{j=1}^{n_A} \left[\sum_{d=1}^{SL} O_{ijd} S_A^{d-\delta_j} \right] A_j = \sum_{j=1}^{n_A} m_{ij} A_j \tag{A.4}$$

$$G_j = \sum_{i=1}^{n_P} \left[\sum_{d=1}^{SL} \Omega_{jid} S_P^{d-d_i} \right] P_i = \sum_{i=1}^{n_P} \mu_{ji} P_i \tag{A.5}$$

The expressions in square brackets

$$m_{ij} = \sum_{d=1}^{SL} O_{ijd} S_A^{d-\delta_j} \tag{A.6}$$

$$\mu_{ji} = \sum_{d=1}^{SL} \Omega_{jid} S_P^{d-d_i} \tag{A.7}$$

are the per capita mutualistic effects of animal j on plant i, and of plant i on animal j respectively, in a year. The cummulative contributions are weighed by the daily survival rates. If the daily survival rates where $S_P = S_A = 1$, then $m_{ij} = \sum_d O_{ijd} = \mu_{ji} = \sum_d \Omega_{jid}$, which is just the total number of days during which the phenologies of i and j coincide.

Following the same argumentation applied to mutualism, we calculate the cumulative costs of intraguild competition for plant i and animal j as follows

$$C_i = \sum_{k=1}^{n_P} \left[\sum_{d=1}^{SL} X_{ikd} S_P^{d-d_k} \right] P_k = \sum_{k=1}^{n_P} x_{ik} P_k \tag{A.8}$$

$$D_j = \sum_{k=1}^{n_A} \left[\sum_{d=1}^{SL} Y_{jkd} S_A^{d-\delta_k} \right] A_k = \sum_{k=1}^{n_A} y_{jk} A_j \tag{A.9}$$

in which the per capita competitive effects experienced by plants and animals are, respectively

$$x_{ik} = \sum_{d=1}^{SL} X_{ikd} S_P^{d-d_k} \tag{A.10}$$

$$y_{jk} = \sum_{d=1}^{SL} Y_{jkd} S_A^{d-\delta_k} \tag{A.11}$$

Thus, the binary overlap matrices $O_{ijd}, \Omega_{jid}, Y_{jkd}, X_{ikd}$ tells us whether or not two species, mutualists as well as competitors, interact on a given day based upon their phenophases. In concert with the aging of the cohort, phenology determines the strenght of the interactions for the whole year. The overlap matrices are three-dimensional arrays containing zeroes and ones. For two interacting species i and j, the entries along the d-axis form an "island of ones" from $d = \max(d_i, \delta_j)$ to $d = \min(\epsilon_i, e_j)$ corresponding to the days of phenophase overlap, flanked by zeroes before and after these dates. Therefore, for the sum defining m_{ij} we can just retain the $O_{ijd} = 1$ terms and drop everything else, such that

$$m_{ij} = \sum_{d=\max(d_i,\delta_j)-\delta_j}^{\min(e_i,\epsilon_j)-\delta_j} s_A^d$$

and similarly for μ_{ji}, x_{ik}, y_{jk}. The resulting expressions are geometric series for which the following identity holds

$$\sum_{d=m}^{n} a^d = \frac{a^{n+1} - a^m}{a - 1} \tag{A.12}$$

if $a \neq 1$;for $a = 1$ the sum is equal to $n - m - 1$. Applying this identity to m_{ij} and μ_{ji}, x_{ik}, y_{jk} we have

$$m_{ij} = \frac{s_A^{\max(d_i,\delta_j)-\delta_j} - s_A^{\min(e_i,\epsilon_j)-\delta_j+1}}{1-s_A} \tag{A.13}$$

$$\mu_{ji} = \frac{s_P^{\max(\delta_j,d_i)-d_i} - s_P^{\min(\epsilon_j,e_i)-\delta_j+1}}{1-s_P} \tag{A.14}$$

$$x_{ik} = \frac{s_P^{\max(d_i,d_k)-d_k} - s_P^{\min(e_i,e_k)-d_k+1}}{1-s_P} \tag{A.15}$$

$$y_{jk} = \frac{s_A^{\max(\delta_j,\delta_k)-\delta_k} - s_A^{\min(\epsilon_j,\epsilon_k)-\delta_k+1}}{1-s_A} \tag{A.16}$$

If $s_A = s_B = 1$, the sum is simply the number of days for which the phenologies of i and j match, e.g. $m_{ij} = \min(e_i, \epsilon_j) - \max(d_i, \delta_j) + 1$. Also, if i and j do not coincide, the formulas above are not valid and $m_{ij} = \mu_{ji} = 0$. A species interacts with itself for a number of days equal to its phenophase, thus the intra-specific competition coefficients are simply

$$x_{ii} = \frac{1-s_P^{e_i-d_i+1}}{1-s_P}, y_{jj} = \frac{1-s_A^{\epsilon_j-\delta_j+1}}{1-s_A}$$

Because inter-specific interactions cannot last longer than any phenophase, it follows that $x_{ii} \geqslant x_{ik}$ and $y_{jj} \geqslant y_{jk}$, i.e. intra-specific is stronger than inter-specific competition.

Yearly recruitment and long-term community dynamics

The number of survivors of a species at the end of its phenophase is a fraction of its initial cohort size. For plants and animals respectively this is

$$\begin{aligned} P_{ie_i} &= P_i s_P^{e_i-d_i} \\ A_{j\epsilon_j} &= A_j s_A^{\epsilon_j-\delta_j} \end{aligned} \tag{A.17}$$

The per capita reproductive ratio of each of these survivors depends positively on the yearly profit due to mutualism and negatively upon the yearly cost of intraguild competition. The number of recruits in the next generation P_i' and A_j' is given by

$$\begin{aligned} P_i' &= P_{ie_i} \times r \exp\left(\frac{F_i}{h_P + F_i} - bC_i\right) \\ A_j' &= A_{j\epsilon_j} \times \rho \exp\left(\frac{G_j}{h_A + G_j} - \beta D_j\right) \end{aligned}$$

in which r, ρ are the per capita reproductive ratios of plants at low densities of mutualists and competitors, and b, β is a scaling factor that converts profits and costs to the same units. The positive dependence given by mutualism is bounded between 0 and 1 by a saturating function with half-saturation constants h_P, h_A. By substituting equations A.4, A.5, A.8, A.9 and A.17 we cast the population dynamics in terms of the number of the abundance of propagules, e.g. seeds or eggs, left by each generation

$$
\begin{aligned}
P_i' &= P_i \times r s_P^{e_i - d_i} \exp\left(\frac{\sum_j m_{ij} A_j}{h_P + \sum_j m_{ij} A_j} - b \sum_k x_{ik} P_k\right) \\
A_j' &= A_j \times \rho s_A^{e_j - \delta_j} \exp\left(\frac{\sum_i \mu_{ji} P_i}{h_A + \sum_i \mu_{ji} P_i} - \beta \sum_k y_{jk} A_k\right)
\end{aligned} \tag{A.18}
$$

Equilibrium, local stability and resilience

Model A.18 is highly non-linear, barring analytical treatment. In our simulations we allowed plenty of time, tipically 3500 years or more, for the community to approach an attractor. An attractor was considered a stable equilibrium if the variance of all population densities over the last 500 time steps of the simulation was less than 10^{-6}. To compute the resilience in cases of stable equilibria, we simply substituted the long-term (i.e. at 3500 days) species abundances P_i^*, A_j^* in the Jacobian matrix of our model,

$$
\mathbf{J} = \begin{bmatrix} \mathbf{J}_{PP} & \mathbf{J}_{PA} \\ \mathbf{J}_{AP} & \mathbf{J}_{AA} \end{bmatrix}
$$

The square block matrices $\mathbf{J}_{PP}$ and $\mathbf{J}_{AA}$ account for the negative effects of competition, and the matrices $\mathbf{J}_{PA}$ and $\mathbf{J}_{AP}$ for the positive effects of mutualism. The elements of $\mathbf{J}$ at equilibrium are

$$
\begin{aligned}
\mathbf{J}_{PP} &= [\delta_{ik} - b x_{ik} P_i^*]_{i,k=1,\ldots,n_P} \\
\mathbf{J}_{PA} &= \left[\frac{m_{ij} P_i^* h_P}{(h_P + \sum_{j=1}^{n_A} m_{ij} A_j^*)^2}\right]_{i=1,\ldots,n_P; j=1,\ldots,n_A} \\
\mathbf{J}_{AP} &= \left[\frac{\mu_{ji} A_j^* h_A}{(h_A + \sum_{i=1}^{n_P} \mu_{ji} P_i^*)^2}\right]_{j=1,\ldots,n_A . i=1,\ldots,n_P} \\
\mathbf{J}_{AA} &= [\delta_{jk} - \beta y_{jk} A_j^*]_{j,k=1,\ldots,n_A}
\end{aligned}
$$

where δ_{ik} (δ_{jk}) is Kronecker's delta: $\delta_{ik} = 1$ if $i = k$ and $P_i^* > 0$, otherwise $\delta_{ik} = 0$ ($\delta_{jk} = 1$ if $j = k$ and 0 otherwise). Resilience was computed as the negative logarithm of the modulus of the leading eigenvalue of $\mathbf{J}$, i.e. $-\log(|\lambda_1|)$, where λ_1 is the leading eigenvalue of $\mathbf{J}$.

Effect of daily survival

We explored the effects of daily survivorship during the period of activity of plants and animals. We ran simulations setting the parameters $S_A = 1.0$, $S_P = 1.0$, hence longer phenology length does not entail a cost under this scenario. We fixed all other parameters to compare with previous simulations ($S_A, S_P = 0.99$) and checked for changes in network structure, diversity and stability. We calculated the coefficients ($m_{ij}, \mu_{ji}, x_{ik}, y_{jk}$) by estimating the temporal overlap between phenologies. For example, if $e_i > e_j$ and $d_i > d_j$ then mutualistic coefficients are $m_{ij}, \mu_{ji} = |e_j - d_i|$ and similarly for competition coefficients (x_{ik}, y_{jk}).

Table 4.1: Effect of daily survivorship on network structure, diversity and stability. Proportion of stable webs (PSW) and average (±sd) of final nestedness (N_f), final connectance (C_f), diversity (n_{total}), mutualistic (M_P) and competitive (X_P) plant interaction strength are reported from a pool of 100 simulations with ($S = 0.99$) and without ($S = 1.0$) daily survival. Parameters used: $r, \rho = 1.5$, $b, \beta = 1.5$, $SL = 18$ days and $T = 3500$ years.

Daily survival	$\overline{C}_f$	$\overline{N}_f$	$\overline{n}_{total}$	$\overline{X}_P$	$\overline{M}_P$	PSW (%)
$S = 0.99$	0.23 ± 0.1	83.5 ± 11.5	20.5 ± 6.7	45.7 ± 4	45.6 ± 14.6	99
$S = 1.0$	0.74 ± 0.1	80.32	47.9 ± 13.8	125 ± 10.9	126.6 ± 39.9	21

The results show that there were no qualitative differences, but only quantitative differences with previous simulations considering survival. More specifically, our results indicate an increase in interaction strength (mutualistic and competitive), connectance, diversity and instability when considering $S_A = S_P = 1.0$, as we expected (see table 4.1).

Phenology distribution

We fitted different distributions of starting dates and phenology lengths of a plant-pollinator community using the data of Pradal et al. (2009). We used the Akaike Information Criterion (AIC) to finally decide which distribution had the best fit to the data. Results indicate that the log-normal distribution had the second best fit for both, plant and animal phenologies (see table 4.2). Moreover, a higher proportion of stable webs was always obtained with log-normally distributed phenologies. Thus, we decided to use log-nomal distributions to generate phenology distributions. Empirical evidence also points to log-normal distributions(Rathcke and Lacey, 1985).

Nestedness

We calculated nestedness in two ways, with the temperature of the interaction matrix (Atmar and Patterson, 1993) and the NODF algorithm (Almeida-Neto et al., 2008). Matrix temperature, T, is a measure of matrix disorder, where nestedness is defined as: $N = (100 - T)/100$. The matrix temperature T ranges from 0° (perfectly nested) to 100° (perfectly non-nested) (Atmar and Patterson, 1993; Guimaraes et al., 2006). The NODF algorithm developed by Almeida-Neto et al. (2008) calculates nestedness metric based on the overlap and decreasing fill algorithm. All nestedness analyses were performed using ANINHADO v. 1.0 (Almeida-Neto et al., 2008). We then calculated relative nestedness as a measure of how nested a network is, compared to the mean expected value from a null model: $N^* = (N - \overline{N}_r)/\overline{N}_r$, where N^* is the relative nestedness and $\overline{N}_r$ is the mean expected value from the null model. The statistical significance of nestedness was estimated using a null model proposed by Bascompte *et al* (2003), where the probability of each cell being occupied is the average of the probabilities of occupancy of its row and column. This means that the probability of drawing an interaction is proportional to the level of generalization of both

the plant and the animal species. Generalist species hence have a higher probability of being assigned an interaction than specialist species (Bascompte et al., 2003).

Mutualistic and competitive connectance

Connectance is the proportion of possible interactions between species that are realized (Jordano et al., 2003). For mutualistic interactions it is defined as $C_M = \frac{L_M}{A*P}$ (Jordano et al., 2003), where L_M is the total number of realized mutualistic interactions in the network and A and P are the total numbers of animal and plant species present, respectively. The connectance of intra-guild competitive interactions was calculated as $C_C^A = \frac{L_C^A}{A*(A-1)/2}$ and $C_C^P = \frac{L_C^P}{P*(P-1)/2}$ for animals and plants, respectively; where L_C^A (L_C^P) are the number of realized competitive interactions for animals (plants).

Asymmetry

The asymmetry of the interaction strength between plants and animals was calculated using relative dependence values (Bascompte et al., 2006). The relative dependence of plant i with animal species j is: $D_{ij} = \frac{m_{ij}}{max(m_{ij},\mu_{ji})}$, where m_{ij} is weighted by the maximum interaction strength value of this plant species with an animal species. In a similar way we can calculate $D_{ji} = \frac{\mu_{ji}}{max(m_{ij},\mu_{ji})}$. We calculated the asymmetry value between plant species i and animal species j as $\Theta_{ij} = \frac{|D_{ij}-D_{ji}|}{max(D_{ij},D_{ji})}$, where $\Theta_{ij} = 1$ means perfect asymmetry and $\Theta_{ij} = 0$ means perfect symmetry of interaction between plant species i and animal species j (Bascompte et al., 2006; Okuyama and Holland, 2008). From this matrix of asymmetries Θ we calculated the mean asymmetry for each species ($\overline{\Theta_i}, \overline{\Theta_j}$).

Disentangling the effects of competition and mutualism

We studied the dynamics of the model with competition and mutualism separately. In this way we can explore the effects in the diversity and stability of the communities when we have: 1) only negative phenological couplings (competitive couplings) or 2) only positive phenological couplings (mutualistic couplings).

Competition only

We ran simulations allowing only interspecific and intraspecific competition, i.e. without mutualistic interactions ($m_{ij} = 0; \mu_{ij} = 0$). The results indicate that competition only can generate high coexistence in all communities with equal variances of starting dates and phenology lengths ($\sigma_p^2 = \sigma_d^2$). These communities are characterized by having many short phenophases and few very long ones, hence having few strong couplings and many weak ones allowing high coexistence. In the lower diagonal (figure A.1) where $\sigma_d^2 < \sigma_p^2$ we find that competition is weaker because starting dates are well spread (few strong couplings); however in the upper diagonal ($\sigma_d^2 > \sigma_p^2$) the opposite occurs: one finds less coexistence because of stronger competitive couplings causing more extinction. The proportion of

Table 4.2: Model selection for plant and animal phenology distributions. No large differences are observed between the fitted models, however log normal distributions produce a higher proportion of stable webs (PSW). Abbreviations used: Negative log likelihood (NLL), Akaike information criteria (AIC), AIC difference (Δ_i), Akaike weight (w_i) and degrees of freedom (*df*). Data of plant and animal phenologies used from Pradal *et al* (2009) Pradal et al. (2009).

Distribution	NLL	AIC	Δ_i	w_i	df	PSW
Negative Binomial	-130.91	268.07	0.0	0.431	2	60 %
Log normal	-130.92	265.83	0.0	0.429	2	97 %
Gamma	-132.01	265.82	2.2	0.140	2	90 %

(a) Animals phenology

Distribution	NLL	AIC	Δ_i	w_i	*df*	PSW
Gamma	-118.34	240.68	0.0	0.550	2	90 %
Log normal	-118.74	241.47	0.8	0.369	2	97 %
Negative binomial	-120.26	244.51	3.8	0.081	2	60 %

(b) Plants phenology

stable communities was 100%. In general we find similar patterns as with the model with mutualism and competition, except for two cases:

1. *High variance communities*: high variance communities under only competitive interactions have higher diversity than high variance communities with mutualistic and competitive interactions (figure A.2). The explanation for this is that under competition and absence of mutualism there can be coexistence of the species (if they are facultative mutualists ($rS^{e-d} > 1$)), but if one or both species engages in mutualistic interactions there can be competitive exclusion by the species with stronger mutualistic interactions, particularly when they have long phenophases which occurs in high variance communities. This means that the effect of mutualism can be very damaging for species coexistence when competition is strong.

2. *LEV communities*: in contrast to high variance communities, *LEV* communities have higher diversity when one allows species to have mutualistic interactions (figure A.2). In the absence of mutualism (only competition) we find a decrease of 30% in the average diversity of the *LEV* communities. Therefore, this is a clear example of the positive effect of mutualism when competition is weak allowing for higher coexistence.

Mutualism only

The diversity is maximally increased if you only consider mutualistic interactions and intraspecific competition, regardless of the phenology distribution. As can be seen in figure A.2, all communities maintain the highest diversity. The proportion of stable communities was 100%.

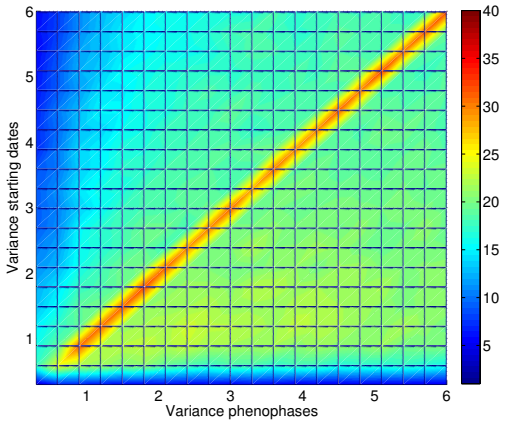


Figure A.1: Final diversity for communities with only interspecific and intraspecific competition ($m_{ij} = 0$, $\mu_{ji} = 0$). Average diversity values shown from 100 simulations for each variance combination. Parameters: $r, \rho = 1.5$, $b, \beta = 1.5$, $S_A, S_P = 0.99$, $SL = 18$ days, and initial community of 60 plant and 60 animal species.

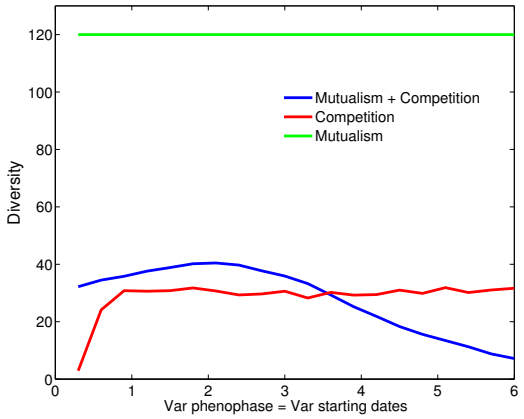


Figure A.2: Effect of mutualism and competition in the diversity of communities. Average values are presented from 100 simulations for the case $\sigma_d^2 = \sigma_p^2$. Parameters: $r, \rho = 1.5$, $b, \beta = 1.5$, $S_A, S_P = 0.99$, $SL = 18$ days, and initial community of 60 plant and 60 animal species.

To conclude, our simulations indicate that mutualism can decrease species coexistence under high intra-guild competition, but it can be highly beneficial for coexistence when competition is weak. Our results from simulations that only allow mutualistic interactions corroborate results from a previous model (Okuyama and Holland, 2008), showing that maximum diversity and stability is always reached. These results show the important role of mutualism and competition (alone or together) shaping these webs and the importance of mutualism for the coexistence of species and in some cases exclusion of species.

APPENDIX B: SUPPORTING FIGURES

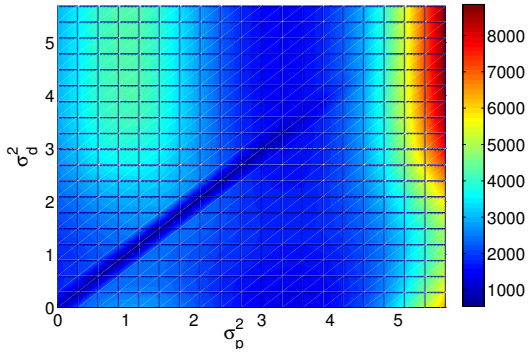


Figure B.1: Mutualistic interaction strength for different phenology distributions. Mutualistic mean interaction strength increases with the variance of phenology lengths. Season length used is SL = 18 days.

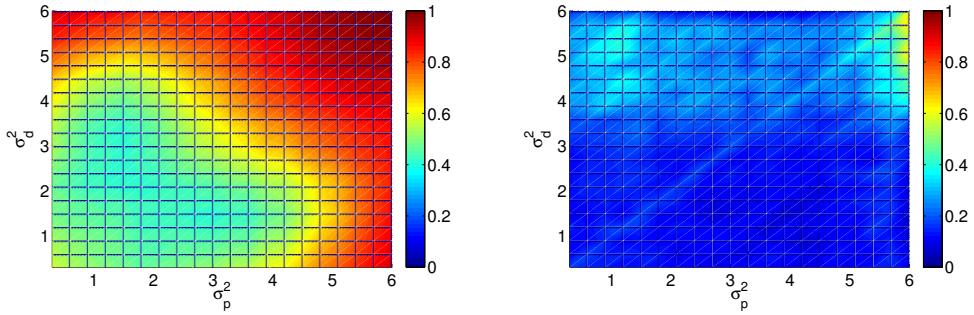


Figure B.2: Changes of intra-guild competitive connectance. a) Initial competitive connectance values. b) final connectance of intra-guild competitive interactions. Results are averaged over 150 simulations for each phenology distribution combination (σ_d^2, σ_p^2). The season length is SL = 18. Initial community diversity was P = 60 and A = 60, for plants and animals, respectively. Demographic parameter values used: $r_i, \rho_j = 1.5$, $b_i, \beta_j = 1.5$ and $S_A, S_P = 0.99$. Changes in competitive connectance are very similar to changes in mutualistic connectance (see figures 1e and 1f), however competition decreases much more than mutualistic connectance.

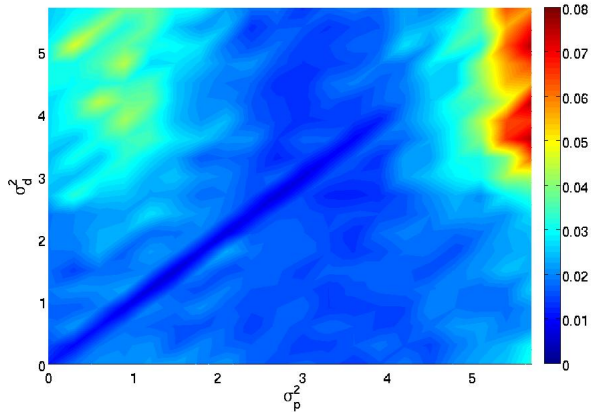
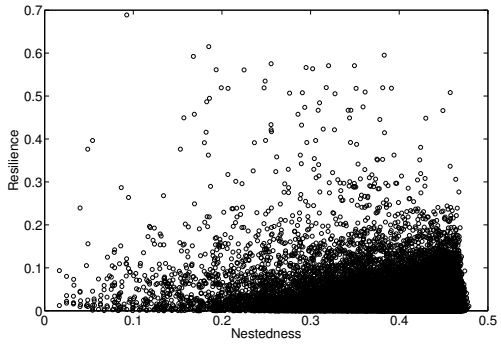
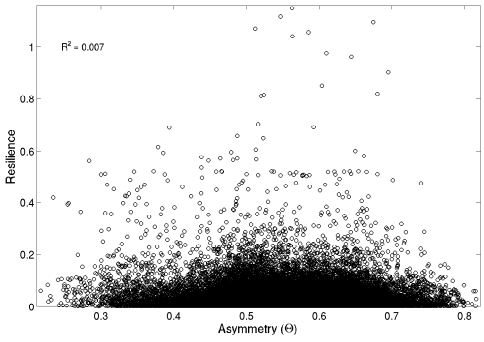


Figure B.3: Resilience for different phenology distributions. Average resilience (return rate) is low for most communities, except for communities with high variance of starting dates and phenology lengths. Season length used is SL = 18 days.



(a)



(b)

Figure B.4: Effect of nestedness and asymmetry on resilience. Each black open circle represents a community from a total of 46,500 network simulations from different phenology distributions and SL = 18 days.

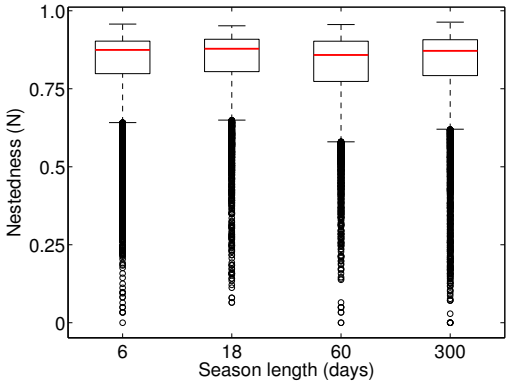


Figure B.5: Effect of season length on nestedness.The black box has lines at the lower quartile, median, and upper quartile values. Red solid lines represent median values and black open circles are outliers. Data from 46,500 network simulations from different phenology distributions and four season lengths ($SL = 6, 18, 60, 300$).

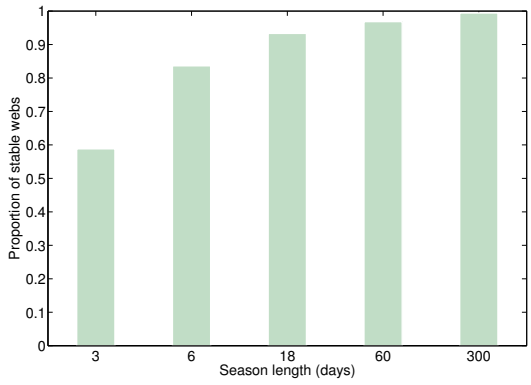


Figure B.6: Proportion of stable webs for different season lengths (SL). Solid green bars represent the proportion of stable webs for each season length. Data from 46,500 network simulations of different phenology distributions and four season lengths ($SL = 3, 6, 18, 60, 300$).

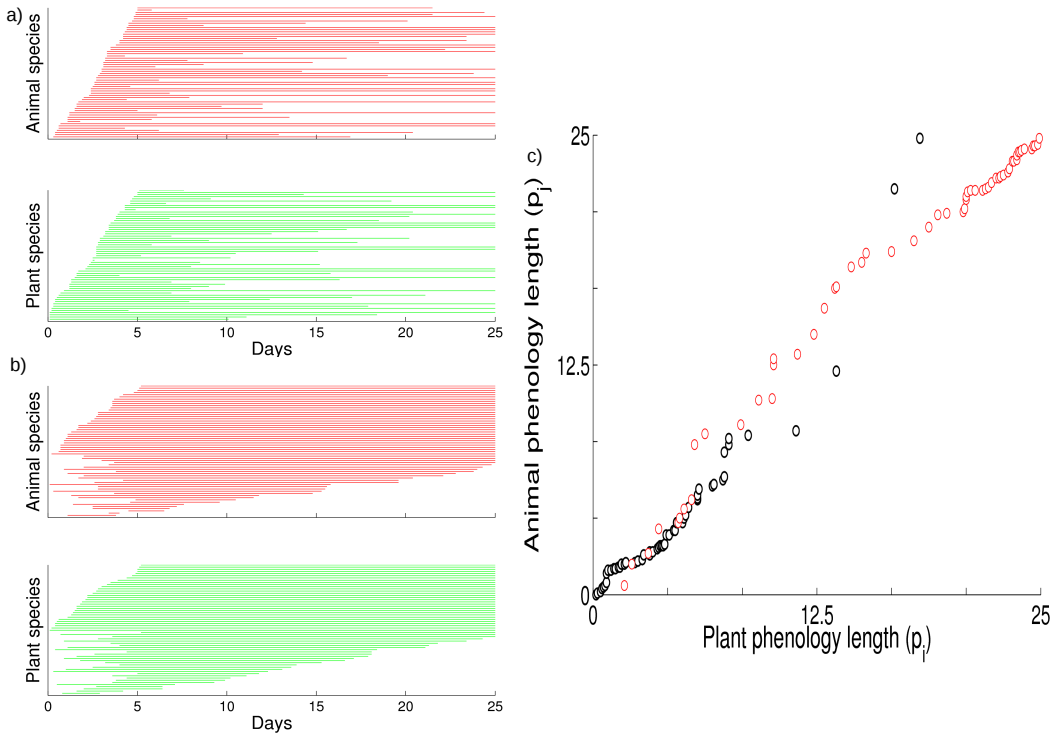
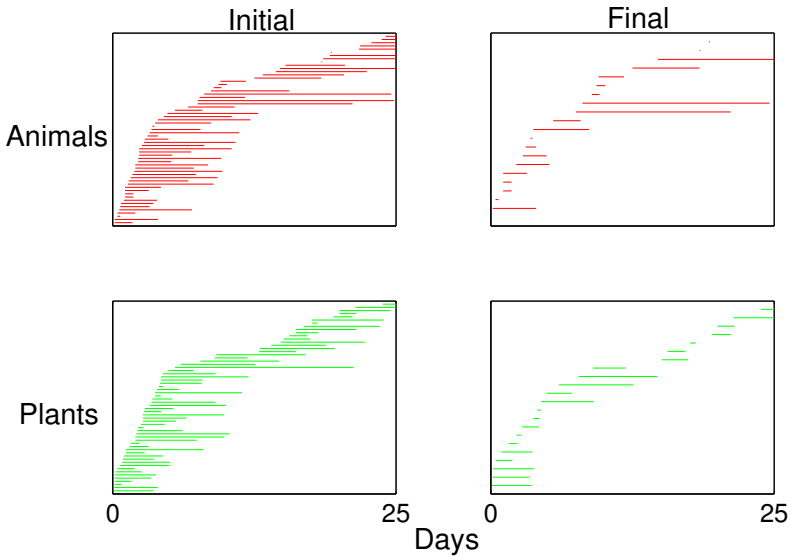
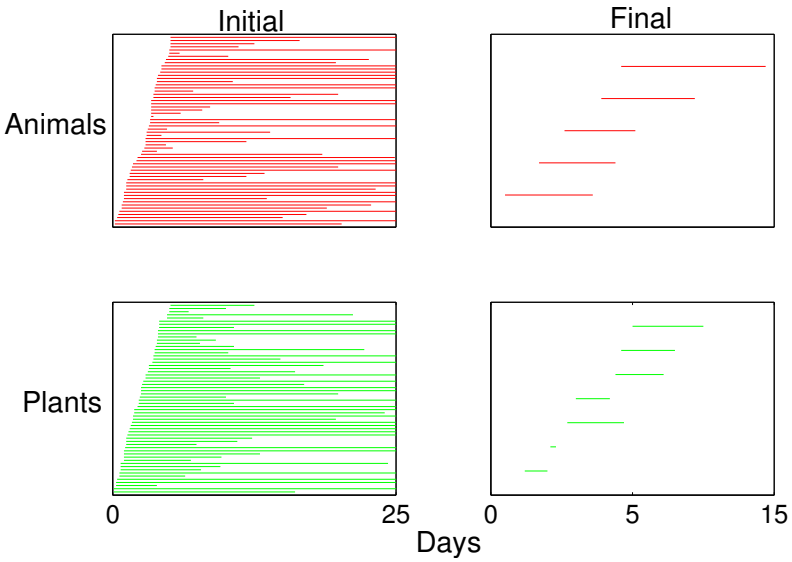


Figure B.7: Phenology distributions of highly overlapping communities. These are communities sampled from a variance of starting dates and phenology lengths $\sigma^2 = 5$. a) Distribution of plant (green solid lines) and animal (red solid lines) phenologies ordered by starting date. b) Distribution of plant and animal phenologies ordered by final date. c) Correlation of phenology lengths (p_i) between plants and animals (p_j) from two different variance values of phenology distributions, $\sigma^2 = 3$ (black open circles) and $\sigma^2 = 5$ (red open circles). Communities with phenologies sampled from a phenology distribution with high variance tend to have large values of phenology length, but *LEV* communities ($\sigma^2 = 3$) tend to have many short phenology length and few large ones.



(a)



(b)

Figure B.8: Changes in phenology distributions of *LEV* and high variance communities. a) *LEV* communities sampled from a variance of starting dates and phenology lengths $\sigma^2 = 3$. b) High variance communities sampled from a variance of starting dates and phenology lengths $\sigma^2 = 5$. Distribution of plant species phenologies are represented in green solid lines and animal species phenologies in red solid lines. All phenologies are ordered by starting dates. Initial phenology distributions are shown in the left panels and final phenology distributions in the right panels. SL = 25 days. *LEV* communities suffered less extinctions and they are characterized by having more spread of starting days and less strong couplings (mutualistic and competitive). On the contrary, high variance communities had more extinctions and stronger couplings (mutualistic and competitive).

5

ARE PLANT-MYCORRHIZAL INTERACTION NETWORKS RANDOM OR STRUCTURED?

Francisco Encinas-Viso, David Alonso, John N. Klironomos, Rampal S. Etienne and Esther R. Chang

The interactions between plants and arbuscular mycorrhizal fungi (AMF) maintain a crucial link between macroscopic organisms and the soil microbial world. These interactions are of extreme importance for the diversity of plant communities and ecosystem functioning. Despite this importance, only recently has the structure of plant-AMF interaction networks been studied using fine taxonomic scale data (i.e. genetic data), suggesting highly structured networks, very similar to plant-animal mutualistic networks, indicative of specific ecological interactions. However, these studies ignore an important feature of plant-AMF interactions: that they occur at an extremely localized scale. Studying plant-AMF networks in a spatial context seems therefore a crucial step. Here, we study the structure of a plant-AMF network using a unique set of spatially explicit species-level data (identified by spore morphology) and a novel methodology. We apply three null models of which only one accounts for spatial effects and we find that the data show no significant differences from null expectations except for those of the spatial null model. Hence, plant-AMF interactions seem to be sufficiently explained by random encounters and dispersal limitation. Thus, contrary to previous findings, we conclude that this plant-AMF network lacks structure: it is not significantly nested or modular. Moreover, we show that not considering spatial structure in the null models could lead to incorrect conclusions about the significance of plant-AMF network structure. Reconciling our results with the contrasting results of others, we argue that the structure of plant-AMF networks depends highly upon the spatial structure and taxonomic resolution of the data.

KEYWORDS: ecological networks, mycorrhizal, nestedness, spatial structure, dispersal

Submitted

INTRODUCTION

Plant-arbuscular mycorrhizal fungi (AMF) interactions are among the best known examples of mutualistic symbiosis (Rosendahl, 2008). AMF are obligate plant-root endosymbionts that colonize approximately two-thirds of terrestrial plant species (Hart et al., 2003). They acquire all their carbon from the host plant and trade it for a range of benefits, notably increased phosphorus uptake (Rosendahl, 2008). Thus, AMF have profound effects on plant community dynamics, diversity and ecosystem functioning (Hart et al., 2003; Rosendahl, 2008). The plant-AMF symbiosis can be highly beneficial, but also detrimental depending on the environmental conditions, developing conditions and even the genotypic-background (Hart et al., 2003; Sanders, 2002). Thus, plant-AMF interactions can range from mutually beneficial (+/+) to mutually detrimental (-/-), passing through neutral (0/0) and commensalistic interactions (+/0) (Johnson et al., 1997a).

Plant-AMF interactions are even more complex because of the different AMF genetic inheritance mechanisms (Sanders and Croll, 2010) and strong spatial structure (Boerner et al., 1996). AMF seem to be highly locally adapted and their dispersal capabilities are limited (Klironomos, 2003; Rosendahl, 2008; Johnson et al., 2012). Some studies show different AMF taxa to be overdominant in different locations, suggesting that the assembly of plant-AMF communities is mainly driven by stochastic processes(Dumbrell et al., 2010a; Lekberg et al., 2012). However, other studies have shown specialization to particular habitats (Opik et al., 2009; Davison et al., 2011) and soil constraints (Dumbrell et al., 2010b), suggesting that niche-driven processes are also relevant in the assembly of plant-AMF communities. A meta-analysis of 19 studies found both neutral and niche-driven AMF communities (Caruso et al., 2012) with roughly half in each category.

Recent studies have suggested that plant-AMF networks are very similar to plant-animal mutualistic networks (Bascompte and Jordano, 2007b); i.e. they are highly nested and modular (Montesinos-Navarro et al., 2012; Chagnon et al., 2012). A significantly nested network shows a pattern wherein specialists interact with proper subsets of the species interacting with generalists (Bascompte et al., 2003) and high modularity means that some groups of species tend to interact more frequently among themselves than with other species (Olesen et al., 2007). However, plant-AMF communities differ from plant-animal mutualistic communities in many biological and ecological aspects. Unlike most animals, AMF are modular organisms (e.g. cnidarians) with flexible morphology that very much depends on environmental conditions contrary to unitary organisms (e.g. insects), where organism structure is predetermined (Pineda-Krch and Poore, 2004). Despite their great flexibility to arrange modules (i.e. iterated units of the organism), once their position is established the spatial relation with neighbors is fixed (Pineda-Krch and Poore, 2004). Therefore, the spatial structure is very important for AMF organism function; for instance, it determines competition, transfer of resources and genetic exchange (Sanders and Croll, 2010; Pineda-Krch and Poore, 2004). In addition, spatial arrangement is even more complex because of the different ways that plants and AMF can be physically connected. One plant may be colonized by several AMF and the belowground hyphal networks of AMF may connect different plant individuals/species, thus allowing exchange of resources between

them (Giovannetti et al., 2004). This spatial complexity should be taken into account when choosing methodologies to assess plant-AMF interactions. Spatial context already seems to be highly important in explaining observed network structure of plant-animal mutualistic webs (Morales and Vázquez, 2008a) that are often much less localized than plant-AMF interactions, so it seems crucial to explicitly consider spatial context when studying the structure of plant-AMF networks.

However, previous plant-AMF studies (Montesinos-Navarro et al., 2012; Chagnon et al., 2012) did not consider spatial context when assessing levels of nestedness and modularity. The aim of this study is to unveil the network structure of plant-AMF in their spatial context. The data set used is spatially explicit and based on presence/absence of plant and AMF species. We test the significance of our observed patterns by using null models. Null models that consider the spatial distribution of species have proven to be important for the analysis of species interactions (Roxburgh and Chesson, 1998). Here we study a null model that incorporates the spatial-autocorrelation of species patterns and we compare it with two non-spatial null models based on complete spatial randomness and environmental filtering, respectively. We use spatial overlap (i.e. species co-occurrence) as a proxy of plant-AMF species interactions and we develop novel metrics to estimate it. This proxy has been used before to describe plant-animal mutualistic networks and plant competition (Roxburgh and Chesson, 1998; Vázquez et al., 2009c). Our study uses AMF species-level data, obtained from morphological characteristics of spores, in contrast with previous studies that used operational taxonomic units (OTUs) of AMF obtained from molecular analysis (Montesinos-Navarro et al., 2012; Chagnon et al., 2012). We find that the data show significant departures from the non-spatial null model expectations, whereas for the spatial null model we find no significant differences. Thus, plant-AMF networks seem to be shaped by random interactions and dispersal limitation; therefore they are strongly affected by local interactions. Our study suggests that considering spatial structure may change the conclusions of previous studies dramatically.

MATERIALS AND METHODS

Collection of plant, AMF and soil data

The study was conducted on a 50 m x 50 m gridded plot that was established at the Long-Term Mycorrhiza Research Site (LTMRS), an old field meadow located in the Nature Reserve of the University of Guelph Arboretum, Guelph, ON, Canada (43°32´30´´ N, 80°13´00´´ W). Sampling points were located at 1m intervals within this grid (51 x 51 points) for a total of 2601 evenly-distributed spatial samples. A more detailed description of the study design and methods is available in Maherali and Klironomos (2012). At each of the 2601 points on the grid we determined the presence/absence of plant and AM fungal species. For plant species presence/absence we used a point-intercept sampling technique (Grieg-Smith, 1983). For presence/absence of AM fungal species, we used trap cultures (detailed method described in Maherali and Klironomos (2012)). In addition, we also measured two abiotic variables (pH and percent organic matter (OM) content of the soil) as described in Klironomos et al. (1993).

Our data set is thus in the form of species presence or absence over a spatially extended grid of dimensions $L = l \times l$. We adopt the following notation: P is the number of plant species, A is the number of AMF species, N_i is the number of cells occupied by plant species i, N_j is the number of cells that are occupied by AMF species j, and n_{ij} is the number of cells where species *i* and *j* spatially overlap (i.e. co-occur). S is the total number of species pairs possible in the matrix.

Null models

To identify significant species interactions derived from the spatial overlap analysis we used three different null models that constitute a range of different constraints. Each null model accounts for different constraints and assumptions. Two null models consider non-spatial effects and one considers spatial effects. One non-spatial null model only accounts for random effects (CSR) (i.e. this the most basic null model (see McGill (2011))) and the other one accounts for environmental filtering (ENV). The spatial null model (SS) accounts for second-order spatial effects. Here is a complete description of each null model:

- Complete spatial randomness (CSR): this null model is the most commonly used and the least constrained one (Gotelli, 2000). This null model randomly reshuffles positions in the grid keeping N_i and N_j fixed without considering spatial second-order effects (i.e. spatial auto-correlation) (McGill, 2011). Biologically, this null model can be interpreted as assuming that there is propagule rain, i.e. immigration is global.
- Environmentally constrained null model (ENV) (Peres-Neto et al., 2001): this null model assumes that environmental conditions control species occurrences. The first step involves calculating the matrix that contains site presence probabilities for each species at each site (i.e. site-specific probability matrix) according to some abiotic constraints. Probabilities of species site presence were generated using logistic regressions, which considers two environmental factors: organic matter content (OM) and pH, to generate a site-by-species matrix containing probability estimates for species presence at each site (i.e. cell) in the spatial plot. The second step involves generating null communities considering the probabilities obtained in the site-by-species matrix.
- Sequential Swap (SS) (Gotelli and Entsminger, 2003): the algorithm of this null model shuffles species positions in the grid while keeping the marginal sum of rows and columns fixed, thus incorporating spatial auto-correlation of the species. In a "sequential swap" algorithm, randomly chosen sub-matrices of the form:

$$\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \text{ or } \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}$$

  are selected, and the cells in the matrix are swapped. Swapping creates a new matrix configuration, but does not alter row and column totals. Swap algorithms always begin with the original matrix and create new matrices by repeated swapping. For each analysis, we used 20000 initial random swaps to remove transient effects.

The sequential swap and the environmentally constrained null model are generally used for biogeographical species co-occurrence data, where the matrix of presence/absence is organized so that sites are in rows and species in columns (Gotelli, 2000). In our case, we applied these null models to randomize species presence across two dimensions (x,y) in the lattice (i.e. spatial plot). For each null model we ran $n = 1000$ simulations, then measured different species co-occurrence metrics and finally, evaluated the statistical significance of the observed co-occurrence against the null distribution of co-occurrences with a non-parametric test (see Appendix A) for each metric and null model. All simulations and statistical tests were developed in R (Team, 2010).

Relationship between range of tolerance (niche) and spatial occurrence

For the environmentally constrained null model, we explored two niche-dimensions: pH and OM. Using these niche dimensions (or range of tolerances) as predictors of species presence, we performed linear regression analysis to check for any correlation between species relative occurrence ($\frac{N_i}{L}$) and range of tolerance. The analysis was applied separately for plant and AMF species.

Measuring Species Spatial Pair-Wise Interactions

Due to the challenges in visually observing cryptic, microscopic organisms that live in the soil, estimating species interaction frequency between plants and AMF are difficult under field conditions. As a proxy for species interaction, we calculated the degree of spatial overlap (i.c. spatial co-occurrence) between plants and AMF species. Thus, we say that two species significantly interact when they spatially overlap (or segregate) more than a random placement model predicts. This approach has been used before to explore other interspecific interactions, such as competition between plants (Roxburgh and Chesson, 1998).

We used three different metrics to estimate various aspects of the interaction between plant and AMF species: 1) Average spatial overlap (F), 2) C-score and 3) Mutual information (I) . Average spatial overlap measures only spatial aggregations between species, while C-score can measure spatial aggregations and segregations. Mutual information also measures spatial aggregations and segregations, but is based on a completely different theoretical framework that comes from information theory. Mutual information has never been used before to estimate species interactions in ecological networks. The use of mutual information for estimating species interactions in ecological networks is novel, to the best of our knowledge. We also calculate two other metrics derived from the mutual information metric (I): Species dependence (D) and asymmetry (A).

1) Average spatial overlap (F)

We developed a simple metric to estimate spatial overlap between species. Two measures of interaction strength can be defined: the fraction of cells for which plant species i is present when AMF species j is also present is: $F_{ij} = \frac{n_{ij}}{N_i}$ and, conversely, the fraction of cells for which AMF species j is present when plant species i is also present: $F_{ji} = \frac{n_{ij}}{N_j}$. Although the

spatial overlap matrix n_{ij} is symmetric, the interaction matrix F is not. Therefore, we define the symmetric interaction strength between plant i and AMF species j as their arithmetic mean: $\hat{F_{ij}} = \frac{F_{ij}+F_{ji}}{2}$ ($F \in [0,1]$). Note that this metric does not consider the asymmetry in overlap of species presences. For example, in the case that $N_i \gg N_j$ and $n_{ij} \approx N_j$, there will be a bias towards the less abundant species (N_j) in the $\hat{F}_{ij}$ value. The maximum value of $F = 1$ is obtained in the symmetric case when there is maximum overlap for both species $F_{ij} = F_{ji} = 1$ and the minimum value when $F_{ij} = F_{ji} = 1/N$. However, highly asymmetric cases ($N_i \gg N_j$, $n_{ij} = N_j$) can also produce high spatial overlap values ($F > 0.5$).

2) C-score

C-score has been extensively used in the biogeographical and ecological literature to estimate species co-occurrence at large geographical scales from presence/absence data (Gotelli, 2000). This metric was first proposed by Stone and Roberts (1990) to calculate species "checkerboard" distributions and is defined as: $C_{ij} = (N_i - n_{ij})(N_j - n_{ij})/S$. We applied this metric to estimate how much plant and AMF species spatially aggregate or segregate in our community. Low values of C means that species aggregate ($C_{min} = 0$) and high values species indicate segregation ($C_{max} = N_i \times N_j$).

*3) Mutual information (*I*)*

We borrowed the concept of *mutual information* (I) from information theory (Ribeiro et al., 2008). This metric measures the mutual dependence of two random variables, to estimate spatial overlap (co-occurrence) between species. Assume that we have a presence(1)/absence(0) data set of species i and j in a spatial plot of dimensions $L = l \times l$. For each species there are four possible states k on each site (cell) of plot L: 1) only species i is present$(1,0)$, 2) only species j is present $(0,1)$, 3) both species are present $(1,1)$ and 4) both species are absent $(0,0)$. Furthermore we define $p(x_i, y_j)$ as the joint probability of species i and j to be in a particular state k . For example, $p(x_i = 1, y_j = 0) = \frac{n_{1,0}}{L}$. Then, $p(x_i)$ and $p(y_j)$ are the marginal probabilities of species i and j, respectively, when present ($p(x_i = 1)$, $p(y_j = 1)$) or absent ($p(x_i = 0)$, $p(y_j = 0)$). Given the spatial distribution of the species, we can estimate the probability of each of these states per species. Calculating these probabilities allow us to measure mutual information between two species X_i and Y_j as:

$$I(X_i; Y_j) = H(X_i) + H(Y_j) - H(X_i, Y_j) \tag{A.1}$$

where

$$H(X_i) = -\sum_{x_i=0,1} p(x_i) \log p(x_i) \tag{A.2}$$

$$H(Y_j) = -\sum_{y_j=0,1} p(y_j) \log p(y_j) \tag{A.3}$$

are the marginal entropies and

$$H(X_i, Y_j) = -\sum_{x_i=0,1} \sum_{y_j=0,1} p(x_i, y_j) \log p(x_i, y_j) \tag{A.4}$$

is the joint entropy of X_i and Y_j. In these expressions$x$ and y are possible outcomes (i.e. presence or absence) of X_i and Y_i, respectively. Substituting Eqs. A.2,A.3 in Eq. A.4 we find

$$I(X_i; Y_j) = \sum_{y=0,1} \sum_{x=0,1} p(x, y) \log \left(\frac{p(x, y)}{p(x)p(y)} \right) \tag{A.5}$$

It can be shown that $I(X;Y) = 0$ if and only if X and Y are independent random variables. We can see this implication in the "if" direction very easily because, by assuming independence, we have $p(x, y) = p(x)p(y)$ and therefore: $\log \left(\frac{p(x,y)}{p(x)p(y)} \right) = 0$. Thus, if there is some dependence, mutual information is always $I(X;Y) > 0$.

In summary, in order to evaluate the mutual dependence of any pair of species, as defined by mutual information, we need the dimension of the lattice, L, the total number of cells occupied by every species, N_i and N_j , and the counts of species i and j in each of the states n_k .

*Species dependence (*D*) and asymmetry (*A*)*

The calculations from mutual information also allow us to estimate species dependence (D) and asymmetry (*A*), which tell us how much species depend on each other (Gorelick et al., 2004) (see Appendix A for details).

Network topology

We explored, for each simulated "null" community, three topological properties commonly studied in mutualistic networks: nestedness, modularity and connectance (see Appendix A for details).

Spatial auto-correlation: Moran's I

We used a Moran's I (I_{Moran})(Moran, 1950) to measure spatial auto-correlation (or second-order effects) in the species spatial distribution. Negative (positive) values indicate negative (positive) spatial autocorrelation. Moran's I ranges from $I_{Moran} = -1$(indicating perfect dispersion) to $I_{Moran} = 1$ (perfect correlation), with $I_{Moran} = 0$ indicating a random spatial pattern (McGill, 2011). We used the R package **"*spdep*"** to estimate Moran's I and species correlograms (i.e. auto-correlation plots) and randomization tests. A correlogram is a graph in which spatial correlation values are plotted, on the y-axis, as a function of the distance classes among the grid cells along the x-axis (McGill, 2011); distance classes here refer to categories of physical distances measured in meters. To test the significance of the spatial autocorrelation we applied a Bonferroni correction.

Table 5.1: Plant-AMF network properties estimated with three different spatial overlap metrics (C-score, F = average spatial overlap, *I* = mutual information) and tested by three different null models (CSR = complete spatial randomness, ENV = environmentally constrained, SS = sequential swap). All metrics approximately estimate the same number of interactions and SS was the most constrained null model. The matrices obtained are based on significant interactions ($p < 0.05$) according to the non-parametric test. None of the network properties were statistically significant ($p > 0.05$) suggesting a lack of structure in this plant-AMF network. .

Metric	*Null model*	*Nestedness*	*Modularity*	*Connectance*
C-score	CSR	25.14	0.54	0.38
	ENV	28.98	0.54	0.27
	SS	0	0	0.01
F	CSR	25.14	0.32	0.36
	ENV	20.36	0.35	0.27
	SS	0	0	0.01
I	CSR	23.74	0.31	0.37

RESULTS

Plant-AMF interactions

All species pair-wise interaction metrics estimated approximately an equal number of significant interactions for each null model (Figure B.1). However, they estimated different numbers of spatially aggregated and segregated interactions (see Appendix B: Table B.1). C-score and average spatial overlap (F) estimated more spatially segregated than aggregated interactions, while mutual information (I) estimated an equal number of segregated and aggregated interactions (Table B.1). In terms of the identity of plant-AMF species interactions, all metrics more or less agree in identifying the spatial aggregation or segregation between plant-AMF species pairs (Figure B.1). Thus, we do not find major differences in the estimations between metrics.

Null models and network properties

The CSR null model predicts the highest number of plant-AMF interactions ($\overline{k} = 100$), followed by the ENV null model ($\overline{k} = 78$). Estimations of network properties using the CSR and ENV null model were similar, regardless of the metrics used: both yielded low nestedness, connectance and modularity (see Table 5.1). This indicates that abiotic factors used in this study (pH and OM) do not seem to determine the level of spatial overlap between plant and AMF species. The SS null model predicts a very low number of plant-AMF interactions ($\overline{k} = 4$): only four plant-AMF species pairs seem to be mutualistically specialized (*H. pratense-S. calospora*, *B. inermis-G. etunicatum, E. vulgare-A.*

morrowiae and *P. pratensis-G. etunicatum*, only one of which, *P. pratensis-G. etunicatum*, shows complete spatial segregation, see Figure B.1). Also, there is almost no network structure in this plant-AMF community (see table 5.1). Figure B.2 clearly shows that SS differs from the other two null models by correctly predicting spatial auto-correlation. Random encounters (interactions) and dispersal limitation seem to explain the spatial co-occurrence of most plant and AMF species pairs.

In the profile of nestedness with different spatial overlap thresholds (i.e. thresholds,f, to assign a plant-AMF interaction) in the observed data we see that nestedness initially increases reaching a maximum and then rapidly decreases (Figure B.3). The profile indicates that nestedness reaches a maximum for a low threshold ($f = 0.15$), which is close to the mean value ($\hat{F} = 0.18$) of the observed spatial overlap distribution (Figure B.4). This indicates that nestedness is mostly associated with the observed positively-skewed species frequency distribution, where there are many species that have low frequencies (or abundances) and hence low chances of interaction and few highly abundant species with many interactions (Vázquez et al., 2007). Moreover, we found no difference between the observed profile of nestedness with those produced by the null models. In fact, the estimated nestedness profiles for the three null models are not significantly different from the observed nestedness profile ($CSR : p = 0.55$, $ENV : p = 0.63$, $SS : p = 0.98$) (Figure B.4). However, the best fit was obtained for the SS null model (Figure B.1) and the CSR and ENV null model tend to underestimate the observed nestedness for low threshold values ($f < 0.2$) (Figure B.1), which suggests that considering spatial-autocorrelation has important consequences. Finally, when we tested the significance of nestedness of matrices obtained from low threshold values ($0.1 < f < 0.35$) with the standard null model to test nestedness (see Bascompte et al. (2003)), which does not consider spatial structure, the results indicated significant nestedness ($p < 0.01$).

Thus, we conclude that: 1) ignoring spatial auto-correlation by using non-spatial null models leads to incorrect conclusions (type I errors) and 2) observed nestedness is not different from the value expected from random encounters. Similar results are obtained when comparing observed connectance and the expected connectance from the null models (Figure B.6). In summary, the plant-AMF network studied here has a very low connectance, nestedness and modularity, which essentially indicates a lack of network structure. The assembly of this community seems to be mainly driven by random interactions, dispersal limitation and species relative frequency. Indeed, plants and AMF have high positive spatial autocorrelation (Figure B.5a and B.5b).Furthermore, the threshold (f) profiles show that high nestedness could emerge from random interactions (CSR) by choosing an arbitrary threshold value (e.g. the mean value of the spatial overlap distribution). Considering spatial structure in the randomization tests is therefore crucial to avoid false-positives.

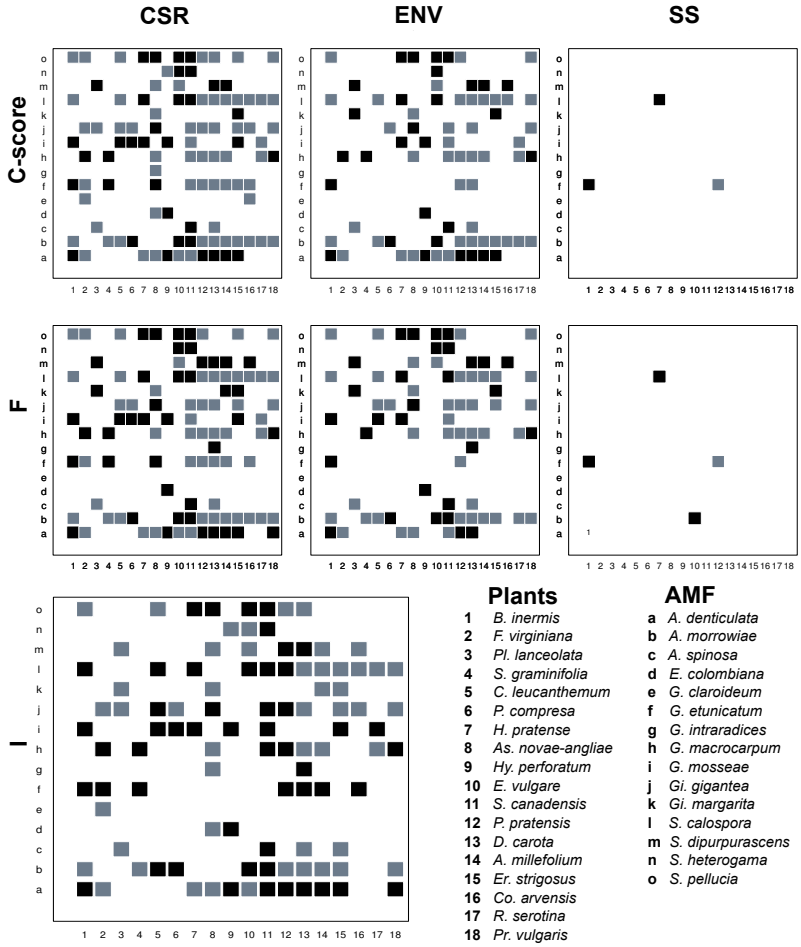


Figure B.1: Plant-AMF interaction matrices using different spatial overlap metrics: C-score, spatial average overlap (F) and mutual information (I). Interaction matrices were obtained for different null models: 1) Complete spatial randomness (CSR), 2) Environmentally constrained (ENV) and 3) Sequential swapping (SS). The interaction matrix based on Mutual information (I) was only applied to the CSR null model (see Appendix A). N =1000 randomizations were applied for each null model. All interactions shown in the figure are statistically significant ($p < 0.05$). Aggregated species are shown in black-filled squares and "segregated" species in gray-filled squares.

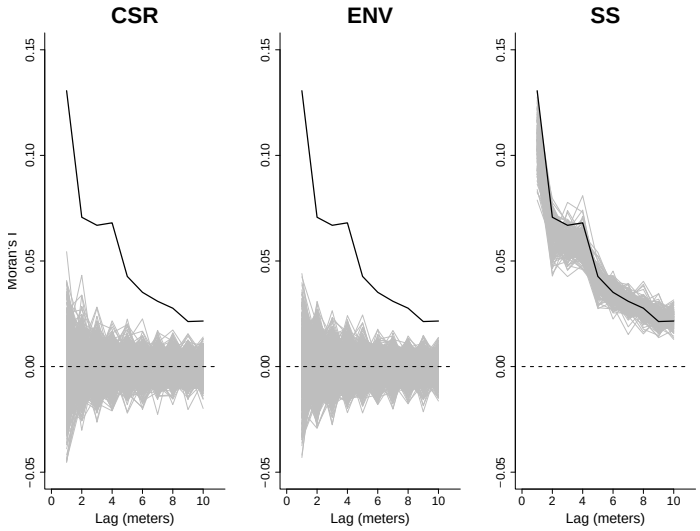


Figure B.2: Correlograms generated by the null models and observed in the plots. Generation of spatial auto-correlation from different null models using the plant species, *Bromus inermis*. 1000 permutations were done to establish the statistical significance of the results. Lag refers to distances in meters. Solid black line = observed data, solid gray lines = simulated values from the null models. The SS null model shows the best fit to spatial auto-correlation seen in field data of *B. inermis*, which is one of a very few plant species found in significant interaction pairings. Other plant and AMF species show similar results. See Table 1 for the key to null model abbreviations.

Abiotic factors and species frequency

Plants and AMF have a significant positive relationship between their relative frequency in the spatial plot and their range of tolerance (i.e. niche width) for pH and organic matter (OM) ($F_{2,15} = 11.74$, $p = 0.0008$, $F_{2,12} = 12.17, p = 0.001$). Thus, plants and AMF with a higher range of tolerance for pH and OM are also those that tend to be more frequent (Figure B.5). This indicates that AMF species, in this community, are distributed across an abiotic gradient, where host plants with the same level of tolerance are also present. However, the ENV null model (based on the logistic regression analysis) does not show any significant effect of pH and/or OM over spatial distribution of the species and their co-occurrence in this community, because ENV null model results are not different from those of the CSR null model.

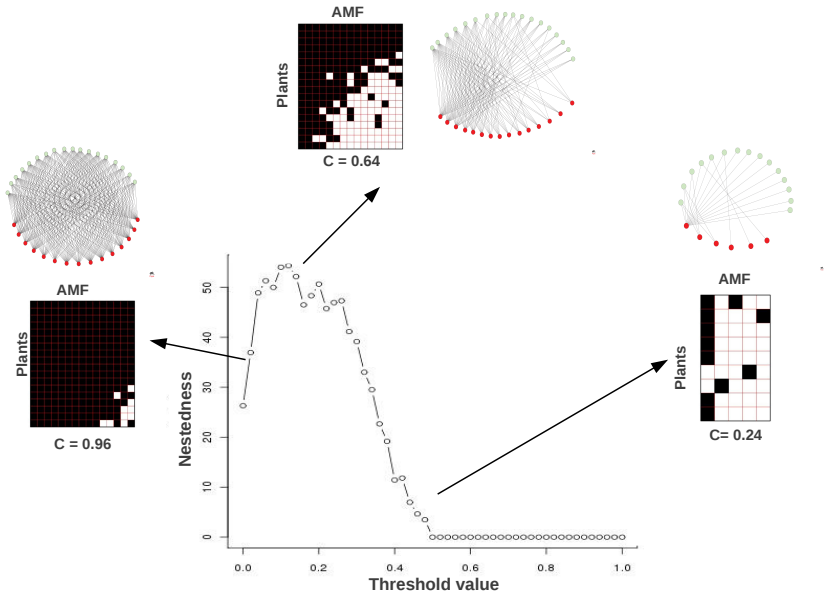


Figure B.3: Profile of plant-AMF network properties across a range of spatial overlap thresholds (f). Spatial overlap thresholds are values of the spatial overlap metric above which co-occurrence is interpreted as a plant-AMF interaction, and below which it is simply a chance event. The central panel shows how nestedness values (black open circles), estimated with the NODF algorithm, change with different threshold values for species spatial overlap. Interaction matrices for three different threshold values (f = 0.05; 0.15; 0.45) are represented in the left, top and right panel, respectively. In plant-AMF interaction matrices each row is a plant species and each column is an AMF species. Black squares show the presence of an interaction between a plant and an AMF species, while white squares show the absence of a plant-AMF interaction. Increasing the threshold values increases the number of interactions and hence connectance. Nestedness reaches an optimum at a low threshold value of co-occurrence (f = 0.15).

Mutual dependence and asymmetry

The estimates of mutual dependence and asymmetry of the observed data indicate that very few species seems to be interacting nonrandomly, confirming previous results from the null model analysis. Also in agreement with the highly aggregated interaction suggested by the null-model analysis, the highest mutual dependence was found for the interaction between *H. pratense* – *S. calospora* ($D_{X,Y} = 0.309$). High mutual dependence was also found for the interactions between *A. nova-angliae* – *G. gigantea* ($D_{X,Y} = 0.284$) and *E. vulgare* – *A. morrowiae* ($D_{X,Y} = 0.225$) (Figure B.3). However, the mutual dependence of the interaction between *B. inermis* and *G. etunicatum* was not high ($D_{X,Y} = 0.052$), in contrast to the null model analysis. Interestingly, the *A. nova-angliae* – *G. gigantea* and *E. vulgare* – *A. morrowiae* interactions also showed very high asymmetry (Figure B.3): spatial information about the

AMF species, *G. gigantea*, tells us more about the spatial distribution of plant species, *A. nova-angliae*, than vice versa ($D_{A.nova|G.giga} = 0.29 > D_{G.giga|A.nova} = 0.17$). and the spatial distribution of *E. vulgare* is more informative about the distribution of *A. morrowiae* than vice versa. ($D_{E.vulga|A.morr} = 0.34 > D_{A.morr|E.vulga} = 0.23$). Furthermore, we did not find any interaction where $D_{X|Y} < D_{Y|X}$. Hence, in general we find that: 1) AMF species distribution provides a similar amount of information or more about the plant species distribution than vice versa and 2) that two AMF species (*G. gigantea* and *A. morrowiae*) rely heavily on the presence of these plant species.

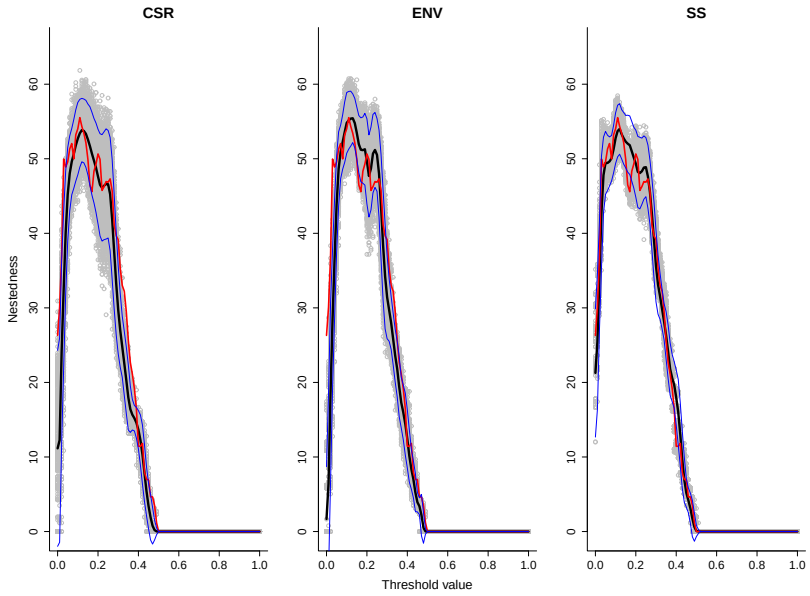


Figure B.4: Changes in nestedness statistics across different spatial overlap thresholds values and for different null models (CSR, ENV, SS): mean nestedness values (black solid line) and the upper (97.5%) and lower (2.5%) quantiles (blue solid lines) from 1000 simulations (grey circles). The red solid line represents observed data.

DISCUSSION

Interactions between plants and AMF species are very complex. They range from parasitic to mutualistic (Johnson et al., 1997a). Although, AMF species seem to be able to colonize different host plants (Sanders, 2002), plants may host specific AMF strains for their own benefit (Kiers and van der Heijden, 2006) and some plant-AMF interactions result in better plant performance than others, especially if they are locally adapted (Klironomos, 2003). Nevertheless, there is evidence suggesting that stochastic processes prevail in the assembly of plant-AMF communities (Dumbrell et al., 2010a; Lekberg et al., 2012). Our study

supports this hypothesis: random encounters and limited dispersal seem to play a major role in structuring plant-AMF communities.

Contrary to previous studies (Montesinos-Navarro et al., 2012; Chagnon et al., 2012), we find that the structure of plant-AMF network is poorly nested and non-modular. The method of AMF species identification might have played a role. We argue below that these contradictory findings are mainly due to the consideration of taxonomic resolution and spatial structure.

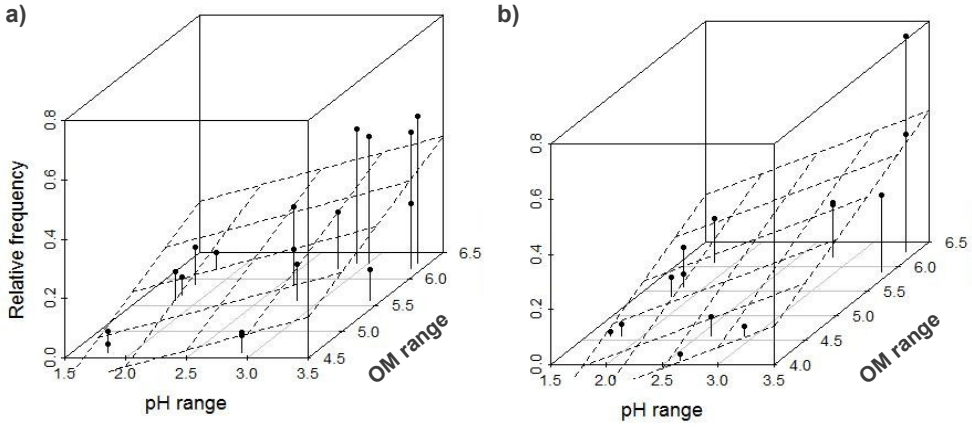


Figure B.5: Plants and AMF with greater niche width are found more frequently. Each stem represents a species with range of tolerance in pH, organic matter (OM) and relative frequency in the spatial plot. The grids shown in (a) and (b) are the regression surfaces obtained from a multiple linear regression taking pH and OM tolerance range as predictors. The analysis was significant for plants ($p = 0.0008$) and AMF ($p = 0.001$). Total number of plant species, $n = 18$, and total number of AMF species, $a = 15$..

Spatial overlaps

Spatial overlap as a predictor of species interaction has been used to test competition among plants (Roxburgh and Chesson, 1998) and to describe plant-animal mutualistic networks (Vázquez et al., 2009c). Vázquez et al. (2009c) showed that spatial overlap, in combination with temporal variation and species abundance, is a good predictor of species interactions. Moreover, a simulation study by Morales and Vázquez (2008a) indicated that spatial aggregation of individuals and limited dispersal strongly affects network statistics (e.g. connectance, nestedness), thus affecting the probability of species interactions.

We obtain very different predictions when incorporating spatial auto-correlation in our null models. More significant species interactions are found when spatial auto-correlation is not

considered. All metrics point out at the high aggregation between the plant species *H. pratense* and the AMF species *S. Calospora*. Empirical evidence from greenhouse experiments indicates that these species interact positively (Klironomos, 2003). In our analysis, the high aggregation of *B. inermis* and *G. etunicatum* seems to be less strong than predicted by mutual dependence; although experimental evidence shows that this association can also be positive (Klironomos, 2003). Our SS null model, which considers spatial auto-correlation, predicts these interactions (*H. pratense-S. Calospora, B. inermis-G. etunicatum*) to be mutualistic. Other positive interactions found by mutual dependence ($D_{X,Y}$) were also found by the CSR and ENV null model, but not by the SS null model (e.g. *A. nova-angliae* – *G. gigantea*), suggesting again that the consideration of spatial structure is important. The high number of negative (segregative) associations found by the CSR and ENV null models and the mutual information metric was not confirmed by the SS null model, which only found one (*P. pratensis-G. etunicatum*). In terms of asymmetry, we found different levels of asymmetric dependence between plants and AMF, which implies that some AMF species depend more on the presence of some plant species and AMF species provide more information about the distribution of their host plants than vice versa. In general asymmetry (A) was low compared to asymmetry estimates in plant-animal mutualistic networks that are based on visitation frequency (Bascompte et al., 2006). Thus, very few interactions were found to be significantly different from our null expectations and we conclude that there is no structure in this community using species-level data of AMF.

Spatial structure and nestedness

Standard null models for mutualistic networks usually use proxies, such as visitation frequency in plant-pollinators webs (Vázquez et al., 2007), for species interactions and most of them ignore the spatial structure of species (but see Vázquez et al. (2009c)). We hypothesized that in plant-AMF interactions we cannot ignore the spatial structure of species because plants and AMF have low mobility and high positive spatial auto-correlation, which greatly affects network structure (Morales and Vázquez, 2008a). Previous studies ignored spatial structure in their analysis using standard null models and concluded that plant-AMF were highly nested (Chagnon et al., 2012; Montesinos-Navarro et al., 2012). In this paper, we showed that only by considering species occurrences (without spatial structure) and by using low threshold values $f < 0.1$ for spatial overlap ($f < 0.1$, meaning that only spatial overlaps lower than 0.1 are considered a product of random spatial distribution, while spatial overlaps higher than 0.1 represent true interactions) we can obtain highly nested networks. Furthermore, these networks are significantly nested when we apply a standard null model to test for significance of nestedness (Bascompte et al., 2003; Almeida-Neto et al., 2008). Accounting for spatial structure makes most of these results insignificant, suggesting standard null models yield incorrect conclusions (i.e. false positives). But, even these standard null models that do not consider spatial auto-correlation (CSR, ENV) show that plant-AMF networks are mostly randomly assembled. Thus, we do not find much evidence of niche mechanisms shaping plant-AMF networks, as previous studies suggested (Chagnon et al., 2012; Montesinos-Navarro et al., 2012). Although some possible specializations in plant-AMF interactions occur (Opik et al., 2009; Rosendahl, 2008),

these interactions seem more labile and variable than those found in plant-animal mutualistic systems. Therefore, invoking niche related processes in plant-AMF networks to explain nestedness must be done cautiously. Stochasticity and dispersal limitation seem a more parsimonious explanation to explain nestedness in plant-AMF networks, given that it is possible to have nested patterns emerging from neutral processes (Krishna et al., 2008).

Taxonomic resolution and modularity

Ribosomal DNA (rDNA) sequence data have allowed the detection of a rich diversity of AMF taxa (Rosendahl, 2008; Koch et al., 2004; Opik et al., 2009). The analysis by Opik et al. (2009) and others (Lekberg et al., 2012) show that several AMF genera form large clusters of AMF operational taxonomic units (OTUs). An example of this diversity is shown by Vandenkoornhuyse et al. (2002), who found 24 OTUs in a single morphologically defined species. Furthermore, the high genetic diversity found is also reflected in high intraspecific functionality (e.g. effect on plant biomass) (Sanders and Croll, 2010).

Recently, Chagnon et al. (2012) and Montesinos-Navarro et al. (2012) showed, using high taxonomic resolution (rDNA sequence data), that plant-AMF networks are highly structured, similar to plant-animal mutualistic webs. Their results show a completely different network structure than our results for species-level data based on spore morphology. This contradiction seems partially explained by scale-dependence (Levin, 1992): taxonomic resolution can greatly affect network structure and some taxonomic scales are better at explaining network structure than others (Eklof et al., 2011). The increased taxonomic resolution in AMF has revealed high genetic diversity (Koch et al., 2004) and highly structured networks (Chagnon et al., 2012; Montesinos-Navarro et al., 2012). However, decreasing resolution leads to an abrupt change in network structure indicating that many plant-AMF interactions seem to be randomly assembled at the species level. We argue that there are two factors that explain these patterns: AMF population genetic and spatial structure.

The population genetic structure of AMF is fairly complex. AMF are haploid asexual organisms in a heterokaryotic state (i.e. their cytoplasm contains several genetically different nuclei) and possess the ability to exchange genetic material between individuals through hyphae fusion (i.e. anastomosis), connecting multiple host plants through the roots (Giovannetti et al., 2001; Sanders and Croll, 2010). Even more interestingly, they show biparental inheritance and segregation of nucleotypes during spore formation (Sanders and Croll, 2010). Experiments have shown that these mechanisms are responsible for the high intraspecific variability of plant-AMF symbiotic effects and these effects can change abruptly in only one generation (Angelard et al., 2010). These studies highlight two important features of plant-AMF interactions: they are highly variable and labile. Furthermore, spatial genetic exchange (through the underground hyphae network) and dispersal limitation can highly constrain plant-AMF OTU interactions (Lekberg et al., 2012). Therefore, we argue that such high modularity is a product of their spatial genetic structure and can only be observed at fine taxonomic resolution.

Montesinos-Navarro et al. (2012) show that grouping AMF OTUs with low genetic dissimilarity (genetic differences $< 1\%$) generates networks with high modularity and low nestedness, and that networks based upon high genetic dissimilarity ($> 1\%$) are less modular, but highly nested. This leads to the following conclusions: 1) the criteria for AMF OTU definition dramatically change plant-AMF network structure, showing again the scale-dependent nature of the problem, 2) AMF genetic structure is unequivocally modular and 3) nestedness can emerge by a 'grouping effect'. This 'grouping effect' means that if one groups more sequences together as OTUs using higher dissimilarity criteria ($> 1\%$), one is also grouping together all the interactions these AMF sequences have with plant species. Consequently one ends up with a nested gradient of 'generalist-specialist' plant-AMF OTUs interactions just by grouping and random sampling effects until reaching a maximum nestedness value as we obtained using spatial overlap data (see Figure B.3). We expect that similar conclusions can be drawn in plant-animal mutualistic networks by considering high resolution data; for example, we could expect less nestedness in individual-based compared to species-level plant-animal mutualistic networks.

Niche-based factors

The predictions of the ENV null model did not differ from the CSR null model, indicating that pH and organic matter (OM) cannot predict the distribution of plant-AMF interactions in this community. Hence, we do not find any clear evidence of niche-related processes based upon abiotic factors in shaping this web, contrary to other studies that have found that pH is an important factor structuring AMF communities (van Aarle et al., 2002; Dumbrell et al., 2010b). However, the regression analysis shows that plant and AMF species frequency in a spatial grid is positively associated with broad ranges of pH and OM. Thus, plants and AMF seem to be spatially distributed according to gradients of pH and OM, although these abiotic factors do not predict how often plant and AMF species interact. Even though the spatial variation of pH and OM was very low in this homogeneous old field site ($\mu_{pH} = 6.8, \sigma_{pH} = 0.5$; $\mu_{OM} = 6, \sigma_{OM} = 1.3$), the frequency of both plant and AMF species still responded significantly to niche breadth. However, species establishment at a specific site may be more influenced by the probability that it reaches that site before a competitor does than by its ability of establishing at that site. Moreover, abundant species are more likely to encounter broader ranges of pH and OM. We argue that a combination of dispersal limitation, competition and priority effects may be stronger than the effect of abiotic niche breadth in this interaction network. But both niche (pH) and stochastic processes might be shaping these communities (Dumbrell et al., 2010b).

Limitations and future directions

Given that our study used presence of freshly produced spores to identify AMF species, we cannot say with 100% confidence that these plants and AMF were interacting. Moreover, AMF sporulation changes depending on physiological and environmental conditions (Redecker et al., 2003). The alternative way to identify AMF species, using pyrosequence data, also has limitations. Firstly, the species concept is still ambiguous for AMF (Heijden

and Scheublin, 2007; Redecker et al., 2003). Given the fluidity in assigning taxa with molecular methods combined with the sensitivity of network parameters to taxonomic resolution, identifying AMF species by spore morphology is more consistent. Secondly, some AMF types can only be detected using spore morphology (but others are only detected using molecular tools (Clapp et al., 2002)). Ideally, a combination of the two methods is needed to cover the whole spectrum of AMF in a community (Heijden and Scheublin, 2007; **?**). Lastly, using molecular sequencing may fail to detect rare species in a sample. This problem is compounded when samples are pooled, as was done in Opik et al. (2009). There is no doubt that the development of next-generation sequencing tools has opened up many new possibilities for studying AMF communities. However, they certainly do not invalidate methods using spore morphology, especially when the sampling effort has been as large-scale and rigorous as in our case (2500 sampling points spread over a grid of 50m by 50m).

We only studied one snapshot of the community and there is evidence showing the importance of phenology and seasonal dynamics in the assembly of AMF communities (Dumbrell et al., 2011). These factors have been shown to be important in shaping plant-animal mutualistic webs as well (Vázquez et al., 2009c) and future studies should therefore consider spatio-temporal variability when investigating plant-AMF networks.

The processes and mechanisms important for the assembly of plant-AMF communities are just starting to be understood (Dumbrell et al., 2010b; Sanders and Croll, 2010). We have shown that dispersal limitation and random interactions seem to be mainly responsible for the observed structure of our studied plant-AMF network. This supports recent evidence that stochasticity is very important in structuring AMF communities (Dumbrell et al., 2010a; Lekberg et al., 2012). We also emphasize the importance of taxonomic resolution and spatial structure in explaining observed patterns of plant-AMF interactions. We hope that our study contributes to reveal the astonishing complexity in the interactions between macroorganisms and soil microbes.

ACKNOWLEDGEMENTS

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APPENDIX A

The complete spatial random placement model

In this appendix section, we give the probability that two species, i and j, co-occur on exactly n_{11} cells given that they are present on N_i and N_j cells from a total set of L cells under the complete spatial random placement null model (CSR, see main text):

$$p(n_{11}|N_i, N_j, L) = \frac{\binom{N_i}{n_{11}}\binom{L-N_i}{N_j-n_{11}}}{\binom{L}{N_i}\binom{L}{N_j}} \tag{A.6}$$

Notice that this probability can only take values between a minimum and a maximum value, $n_{min} = max(0, N_i + N_j - L)$ and $n_{max} = min(N_i, N_j)$, respectively. It can be checked that:

$$\sum_{n_{11}=n_{1,1}^{min}}^{n_{1,1}^{max}} p(n_{11}|N_i, N_j, L)$$

We test the validity of our theoretical distribution by monte carlo simulations ($n = 10000$) applying simple random reshufflings to species positions in the spatial grid, as it will be described in the *complete spatial random* null model (CSR). Our monte carlo simulations confirmed our theoretical expectations.

In sum, if we characterize the spatial distribution of a pair of species, i and j, only by their absolute frequencies on a set of L cells, i.e., N_i and N_j , respectivevly, we know how the variable n_{11} is distributed under the complete spatial ramdom placement nul model (CSR), i.e., under the assumption that N_i and N_j will be placed at random on the set of L cells. Therefore, any statistic,χ, that can be written as a function of these quantities, such as, the C-score, the average spatial overlap ($\mathcal{F}$), and the mutual information (I), has a theoretical distribution under the CSR model that can be exactly calculated by using Eq. (A.6). In particular, the expected value of χ, under the CSR model, for a pair of species characterized by N_i , N_j absolute abundances on set of L cells, is, accordingly:

$$\overline{\chi} = \sum_{n_{11}=n_{1,1}^{min}}^{n_{1,1}^{max}} \chi(N_i, N_j, n_{11}) p(n_{11}|N_i, N_j, L)$$

The non-parametric test of significance

If we know the theoretical distribution of a statistic, we can set up an exact significance test. Since this is only possible for one of the three models we have analyzed (CSR, see previous section), for the sake of comparison, we have opted instead for applying the same non-parametric test, based on model simulated data, for all our models.

From our simulated data produced by each model we calculated the upper (97.5%) and lower (2.5%) quantiles of the distribution our statistic at work. This assumes a confidence level of $\alpha = 0.05$. Finally, we estimated the significant interactions comparing whether our observed statistic falls or not the tails of the simulated distribution.

Appendix A

*Species dependence (*D*) and asymmetry (*A*)*

The calculations from mutual information also allow us to estimate a normalized measure of how much species depend on each other (Gorelick et al., 2004). For example, we can infer how much knowledge of the spatial distribution of species Y tells us about the spatial distribution of species X and vice versa:

$$D_{X|Y} = \frac{I(X,Y)}{H(Y)} \tag{A.7}$$

$$D_{Y|X} = \frac{I(X,Y)}{H(X)} \tag{A.8}$$

where $H(X)$ and $H(Y)$ are the marginal entropies of X and Y, respectively; and the symmetric or mutual dependence can be estimated as:

$$D_{X,Y} = \frac{I(X,Y)}{\sqrt{H(X)H(Y)}} \tag{A.9}$$

Therefore, this metric tells us how much species X and Y depend on each other. There can be differences between $D_{X|Y}$ and $D_{Y|X}$, which are asymmetric cases of the information provided by the spatial overlap of the species. For example, when $D_{X|Y} > D_{Y|X}$ the information gained from species Y for the spatial distribution of species X is more than that inferred from species X about the distribution of species Y. Thus, we can define this asymmetry of mutual information as: $A_{XY} = |D_{X|Y} - D_{Y|X}|$. This is another way of describing the extent to which species depend on each other. A high value of A_{XY} indicates that one of the species depends more on the presence of their mutualistic partner than the other. This is similar to the definition of asymmetry used in plant-animal mutualistic networks (Bascompte et al., 2006). We computed $D_{X|Y}$, $D_{Y|X}$, $D_{X,Y}$ and A_{XY} for our data.

Network topology

*Nestedness (*N*)*

Nestedness describes a non-random pattern of species interactions where specialist species interact with proper subsets of more generalist species (Bascompte et al., 2003). We estimated nestedness using the NODF algorithm developed by Almeida-Neto et al. (2008) because of its statistical robustness. NODF is based on standardized differences in row and column fills and paired matching of occurrences. We used the R package *"bipartite"* to estimate NODF (Dormann et al., 2009)

Because nonzero spatial overlap between two species does not necessarily mean that an interaction exists, we introduced an interaction matrix M (with elements m_{ij}) that is derived from the observed spatial overlap matrix F in the following way. We assumed a threshold spatial overlap f above which overlap is assumed to indicate an interaction, and below

which the overlap is assumed to be coincidental, i.e. not due to an actual interactions. In formula, the interaction matrix between plant species i and AMF species j is computed as:

$$\begin{cases} m_{ij} = 1 & \text{if } \hat{F}_{ij} > f \\ m_{ij} = 0 & \text{if } \hat{F}_{ij} < f \end{cases}$$

We computed these interactions matrices for the observed network and for the networks resulting from the randomizations in the different null models, and tested the significance of our observed data with a t-test. We did this for a range of thresholds $f \in [0, 1]$, thus creating a profile. We also tested the nestedness significance of the interaction matrices computed from different spatial overlap thresholds (f) with a standard null model used to test the significance of nestedness: the PRC (Probable Rows and Columns) model (Bascompte et al., 2003). PRC is a null model that assumes that the probability of a species interaction between species i and j is: $(P_i/N_c + P_j/N_r)/2$, where P_i is the number of presences in row i, P_j is the number of presences in column j, N_c is the number of columns, and N_r is the number of rows.

Modularity (M)

A modular network consists of interconnected modules. Each module is formed by a group of species, which are more connected to one another than to species in other groups (Olesen et al., 2007). We used the *simulated annealing* algorithm (SA) provided by R. Guimerà (Guimerá and Amaral, 2005) to estimate the level of modularity (M). Basically, M is a measure of the extent to which species have more links within their modules than expected if linkage is random.

Connectance (C)

Connectance measures the proportion of realized interactions (i.e. links) among all possible interactions in a network and is defined as $C = \frac{k}{P*A}$, where k represents the number of realized interactions between plant and AMF species and P and A represent the number of plant and AMF species present, respectively, in the network (Jordano et al., 2003).

APPENDIX B: SUPPORTING FIGURES AND TABLES

Table B.1: The number of significant plant-AMF interaction pairs identified by three different metrics (C-score, *I* and F). Aggregation refers to pairs that tend to co-occur together (i.e. spatially overlapped) and segregation refers to pairs that tend to not co-occur together. All interactions are significant ($p < 0.001$)

Metric	Aggregation	Segregation	Total
C-score	37	66	103
I	52	49	101
F	43	55	98

Table B.2: I_{Moran} spatial auto-correlation estimations for plant and AMF diversity. 1000 randomizations were applied to test the significance of the observed spatial distribution. p-values were calculated using a double-sided probability ($\alpha = 0.05$) and a Bonferroni correction was applied to correct for multiple tests Significant codes: $p < 0.01$ $**$, $p < 0.001$ $***$

Lag	I_{Moran}	$\pm SD$	Pr(I)	p
1	2.66e-01	1.96e-04	19.02	***
2	2.31e-01	1.0e-04	23.05	***
3	2.08e-01	6.92e-05	25.06	***
4	1.88e-01	5.32e-05	25.93	***
5	1.59e-01	4.37e-05	24.11	***
6	1.23e-01	3.73e-05	20.27	***
7	0.94e-01	3.28e-05	16.55	***
8	0.57e-01	2.94e-05	10.71	***
9	0.4e-01	2.68e-05	7.98	***
10	0.26e-01	2.47e-05	5.35	***

(a) Correlogram of AMF richness

Lag	I_{Moran}	$\pm SD$	Pr(I)	p
1	2.19e-01	1.96e-04	15.66	***
2	1.04e-01	1.00e-04	10.43	***
3	0.94e-01	6.91e-05	11.35	***
4	0.64e-01	5.32e-05	8.94	***
5	0.4e-01	4.36e-05	6.13	***
6	0.32e-01	3.73e-05	5.37	***
7	0.36e-01	3.28e-05	6.43	***
8	0.19e-01	2.94e-05	3.62	***
9	0.19e-01	2.68e-05	3.81	***
10	0.15e-01	2.47e-05	3.12	**

(b) Correlogram of plant richness

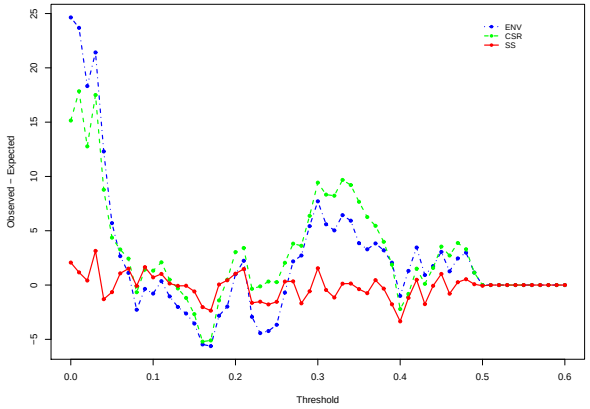


Figure B.1: Differences between observed and expected values of nestedness vs. threshold values (f) of spatial overlap for the three null models (shown here in different colors).

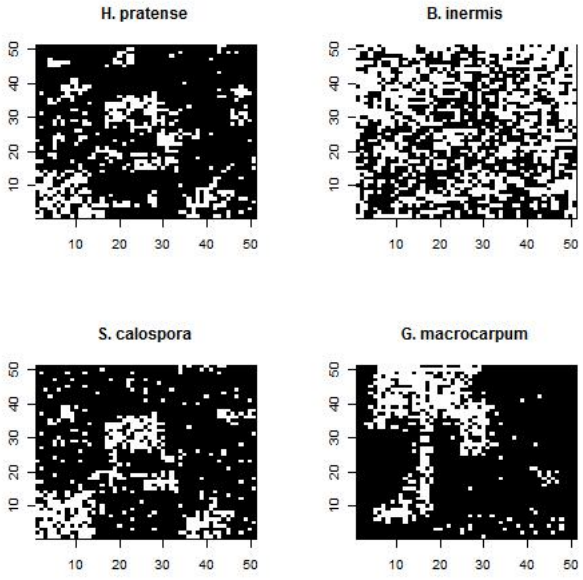


Figure B.2: Spatial distribution of plants and AMF. Black squares represent species absence and white squares represent species presence. X and Y axes show the distance in meters. Plants and AMF show different degrees of spatial auto-correlation.

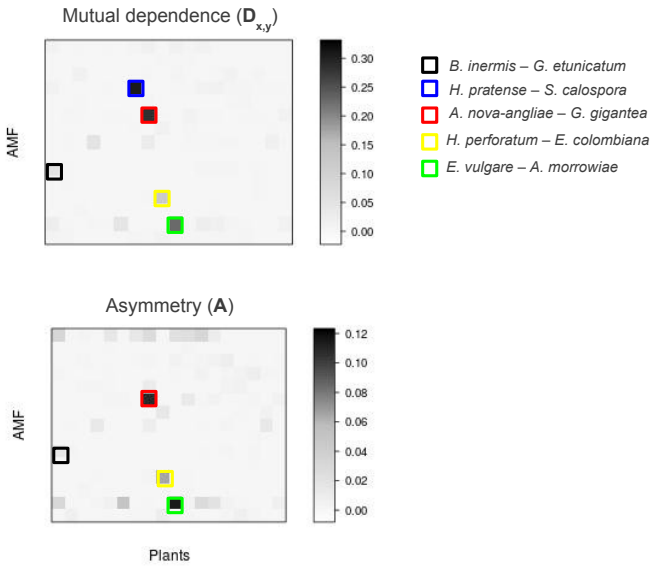


Figure B.3: Asymmetry (A) and mutual dependence ($D_{x,y}$) between plant and AMF species based on mutual information (I). Darker grey indicates greater mutual dependence and asymmetry. Interaction pairs are highlighted by different colors. Few plant-AMF interactions have high mutual dependence and asymmetry. The interactions that are highly asymmetrical are also highly mutually dependent

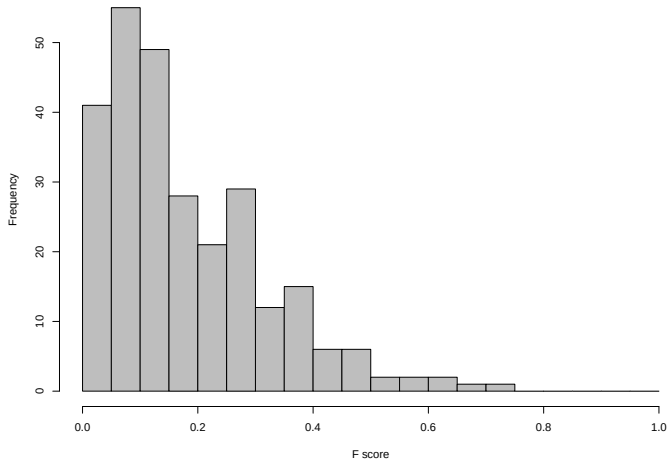
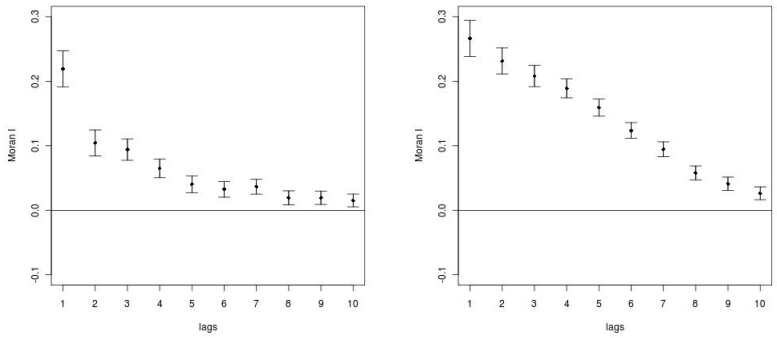


Figure B.4: Histogram of observed spatial overlap values (F) of plant-AMF community. The histogram shows a positively skewed distribution of plant-AMF spatial overlaps from the studied community. Total number of plant species,$n = 18$, and total number of AMF species, $a = 15$.



(a) Plants (b) AMF

Figure B.5: Correlograms of plant (a) and AMF (b) diversity across the spatial plot. We applied 1000 permutations to test the statistical significance of the results. Lag refers to distances in meters. For all lags the test was significant ($p < 0.001$). The plots show positive spatial autocorrelation ($I_{Moran} > 0$) for both guilds across different distances (lags).

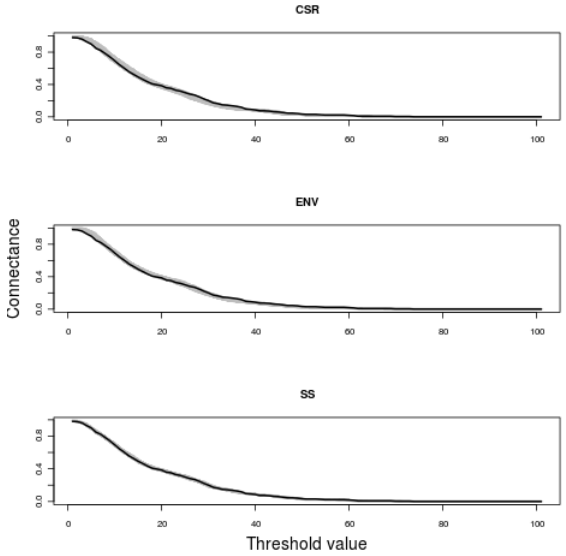


Figure B.6: Changes in connectance across different spatial overlap threshold values and for different null models (CSR, ENV, SS). Connectance values from 1000 simulations (grey thin solid lines) and observed F (black solid line) are shown for each threshold value of species spatial overlap f. Connectance decreases with increases of threshold values for spatial overlap.

6

EMERGENCE OF COMPLEMENTARITY AND CONVERGENCE FROM BASIC PROCESSES

Francisco Encinas-Viso, Carlos J. Melián and Rampal S. Etienne

Plant-animal mutualistic networks are highly diverse and structured. This has been explained by coevolution through niche based processes. However, this explanation is only warranted if neutral processes (e.g. limited dispersal, genetic and ecological drift) cannot explain these patterns. Here we present a spatially explicit model based on explicit genetics and quantitative traits to study the connection between genome evolution, speciation and plant-animal network demography. We consider simple processes for the speciation dynamics of plant-animal mutualisms: ecological (dispersal, demography) and genetic processes (mutation, recombination, drift) and morphological constraints (matching of quantitative trait) for species interactions, particularly mating. We find trait convergence and complementarity and topological features observed in real plant-animal mutualistic webs (i.e. nestedness and centrality). Furthermore, the morphological constraint for plant reproduction generates higher centrality among plant individuals (and species) than in animals, consistent with observations. We argue that simple processes are able to reproduce some well known ecological and evolutionary patterns of plant-animal mutualistic webs.

KEYWORDS: mutualism, convergence, complementarity, coevolution, plant-pollinator interactions, drift

INTRODUCTION

Since Darwin's book "On The Origin of Species" (Darwin, 1862b), the idea of coevolution [1] has sparked interest from biologists trying to understand how species interactions generate trait changes. The first clear indication of coevolution was Darwin's moth example (**?**) showing that the long corolla from the orchid *Angraecum sesquispedale* could only be reached by a pollinator species with a similar proboscis length. However, much later Janzen (1980) argued that this amazingly high specialization between plants and animals was not the only example of coevolution. He explained that coevolution can also be the product of multiple-species interactions, a term that he coined "diffuse coevolution". Diffuse coevolution means that selection on traits is determined by the interaction of all species in the community and not only based on pair-wise interactions. This is based on the idea of pollination or dispersal "syndromes", where plants have a set of traits that attract a specific group of pollinator or animal seed-disperser species.

Later, the idea of "diffuse coevolution" was related to patterns of nestedness detected across biogeographic regions in mutualistic networks. Nestedness, defined as a non-random pattern of interactions where specialist species interact with proper subsets of more generalist species puts the concept of "diffuse coevolution" in a more quantitative context (Bascompte et al., 2003). Nestedness patterns have been shown to provide information about the underlying network dynamics. For example, nestedness is associated with stability and coexistence of species in a community (Bastolla et al., 2009; Okuyama and Holland, 2008).

Several studies have modelled coevolutionary dynamics in mutualistic systems of a few species (Ferriere et al., 2007; Law et al., 2001; Ferdy et al., 2002; Gomulkiewicz et al., 2003; Jones and Ferrière, 2009), particularly highly specialized (i.e. obligatory mutualists) systems of plant-animal interactions, such as the fig-fig wasp mutualism (Bronstein et al., 2006). These studies have determined the ecological conditions for coevolutionary stable systems (i.e. coESS) (Jones and Ferrière, 2009; Law et al., 2001). However, more complex cases of evolution involving multispecific interactions in the context of quantitative genetics and explicit speciation mechanisms remain unexplored.

There are two trait-based patterns in plant-animal mutualistic networks that provide evidence for niche-driven and coevolutionary processes shaping these webs: evolutionary *complementarity* and *convergence* (Bascompte and Jordano, 2007b). Complementarity describes that there is selection for trait matching between plant and animal traits (e.g. corolla length-proboscis length, frugivore body mass-seed size) (Rezende et al., 2007a; Bascompte and Jordano, 2007b). Therefore, complementarity seems the clearest explanation of reciprocal evolution (i.e. coevolution). Convergence is consistent with observed trait similarity among evolutionarily distantly related species of the same guild (e.g. pollinators with similar proboscis length) and is assumed to be caused by selective pressures and developmental constraints (Bascompte and Jordano, 2007b). Evolutionary convergence in plant-animal mutualisms partly explains the formation of 'syndromes' produced by the presence of specific mutualist partner species (Bascompte and Jordano, 2007b; Howe and Smallwood, 1982a; Waser et al., 1996). For example, plant species with a specific corolla morphology may determine the evolutionary convergence of pollinator species traits (**?**). Guimaraes et al.

1 defined as reciprocal evolutionary change between species

(2011) studied a coevolutionary model of mutualistic webs where selective pressures came only from mutualistic partners and found that coevolution promotes complementarity and convergence supporting the idea that selection through niche-driven mechanisms (i.e. the biotic environment) is mainly responsible for the observed patterns. However, non-selective causes can also produce evolutionary convergence (Losos, 2011).

Krishna et al. (2008) and Canard et al. (2012) have shown that random fluctuation of species abundance (i.e., ecological drift) can explain some of the topological properties in mutualistic and trophic webs, respectively. These studies do not take into account explicitly the genetics of quantitative traits and speciation dynamics. The question then arises whether models that describe quantitative trait dynamics with explicit genetics and speciation in the context of random fluctuations of species can generate simultaneously the evolution of convergence, complementarity and network topology observed in real plant-pollinator webs.

Recently, various neutral eco-evolutionary models have started to consider genetics explicitly and more realistic assumptions about the speciation process (de Aguiar et al., 2009; Melián et al., 2010, 2012). These models, which consider intraspecific variation and explicitly incorporate three of the main evolutionary forces (mutation, recombination and drift (Lynch, 2007; **?**)), can predict biodiversity patterns well. Furthermore, these models use a common theoretical framework based on the neutral theories of evolution (Kimura, 1983) and ecology (Hubbell, 2001). They allow testing model predictions with available data on diversity, species traits, spatial distribution and genetics. The progress in this area is rapid, but it is still in its early stages.

Here, we develop an individual-based stochastic model of plant-pollinator interactions that considers explicit genetics, phenotype expression and spatial structure of sexually reproducing individuals, to study the eco-evolutionary dynamics of plant-pollinator webs. We find emergence of plant-pollinator network topological properties such as nestedness and centrality, and the evolution of trait convergence and complementarity. We argue that basic ecological and genetic processes in combination with physical constraints of plant-pollinator interactions, can generate observed plant-pollinator network topology and the evolutionary patterns of plant-pollinator traits.

THE MODEL

We consider the eco-evolutionary dynamics of plants (P) and animal pollinators (A). These two guilds interact mutualistically: plants need the presence of pollinators and vice versa to reproduce. Hence the mutualism is obligatory for both partners.

General eco-evolutionary dynamics

Our model is a stochastic individual-based model with overlapping generations and zero-sum birth-death dynamics. The population consists of J_P and J_A haploid gonochoric (i.e. separated sexes) individuals for plants and animals, respectively; with explicit binary genomes of size L. Each plant and animal population reproduces sexually and is spatially structured. The reproduction of each guild is done in turns (i.e. asynchronically). The individual-based events occur in the following order: an individual is randomly selected to

die and then a female individual is randomly chosen among all females within a distance $d_{max}$of the dead individual's position to mate. Thus, death and reproduction events only occur at a local scale to reflect limited dispersal.

There are two conditions for sexual reproduction: 1) the geographic distance d_{ij} between two individuals (plant or animal), a female i and a male j, from the geographic distance matrix D has to be lower than the maximum geographic distance d_{max} ($d_{ij} < d_{max}$). In case there are no potential mates, a different female is randomly chosen until a potential mate is found. We have two geographic distance matrices: D^P and D^A for plants and animals, respectively. 2) the genetic similarity q_{ij} between two individuals (defined below) has to be higher than the minimum genetic distance q_{min} to be able to mate and leave viable offspring (hence individuals mate assortatively). The genome of each individual is represented by a sequence of L loci, where each locus can be in two allelic states, $+1$ or -1 . Each individual i in a population of size J is represented as a vector: $S^i = (S^i_1, S^i_2, ..., S^i_L)$, where S^i_u is the u^{th} locus in the genome of individual i. The genetic similarity between individuals is calculated as the sum of identical loci across the genome:

$$q_{ij} = \frac{1}{L} \sum_{u=1}^{L} S^i_u S^j_u \tag{A.1}$$

where $q_{ij} \in \{-1, 1\}$. The offspring born from this mating is dispersed within the geographic distance,d_{max} , and will occupy the geographic position of the just deceased individual.

The genome of the offspring is obtained by a block cross-over recombination of the female genome S^i and male genome S^j, where a locus l in the genome of the parents is randomly chosen partitioning the genome of each individual in two blocks. All genes beyond that locus l in either organism genome are swapped between the two parents and eventually form two new genomes. One of the two new genomes is randomly chosen from a uniform distribution for the offspring. The offspring's genome undergoes mutations at mutation rate μ. Figure B.1 describes the model, including the recombination-mutation process.

At the beginning of the simulations all individuals are genetically identical ($q = 1$), hence they are all able to mate with one another. We can visualize the genetic similarity between individuals of a guild as an evolutionary spatial graph (Melián et al., 2010), where nodes correspond to individuals and the length of edges correspond to the geographic distance between a pair of genetically similar ($q_{ij} > q_{min}$) individuals. At the beginning of the simulation this leads to a fully connected graph under an evolutionary process with mutation, recombination and dispersal. The connectance of the graph will decrease when species are formed (i.e. speciation). Here, we define a species as a group of genetically related individuals, where two individuals in sexual populations can be conspecific while also being incompatible, as long as they can exchange genes indirectly through other conspecifics. This is the definition of 'ring species' (de Aguiar et al., 2009; Melián et al., 2010).

The speciation process in this model is similar to previous neutral speciation models with explicit genetics (de Aguiar et al., 2009; Melián et al., 2010). Individuals become more and more genetically divergent through the mutation and recombination process and the spatial segregation. This will finally produce the formation of two genetically incompatible clusters of individuals, i.e. two species. This speciation process, also called 'fission-induced'

speciation (Melián et al., 2012), goes on with the formation of more clusters and genetic divergence between individuals of different species. However, the diversification dynamics will fluctuate due to random extinctions (death of last individual of a species). A stochastic balance between speciation and extinction is eventually reached giving the final steady-state of the metacommunity (Melián et al., 2012).

Quantitative traits

The quantitative trait (z) of each individual is determined by additive genetic effects of the genome (g) (i.e. no epistasis) plus a normally distributed environmental effect (ϵ)($\mu_\epsilon = 0, \sigma^2_\epsilon = 1$). Thus, $z_i = g_i + \epsilon$ determines the phenotype or quantitative trait (z_i) of each individual. The genetic component (g_i) of an individual i is:

$$g_i = L + \sum_{u=1}^{L} S^i_u \tag{A.2}$$

calculated as the sum of alleles across the genome (Kondrashov and Shpak, 1998) plus the number of loci to avoid negative trait values. If we sample genomes of size L from a uniform distribution, the distribution of genetic values would have mean L and a variance given by the algebraic sum of allelic values. We assume two quantitative traits, one for each guild: proboscis length (z^A_i) in pollinators and corolla length (z^P_i) in plants.

Phenotypic similarity

We measured phenotypic similarity between individuals of the same guild and of different guilds to study the relationship between genotypic and phenotypic similarity. The phenotypic similarity (p_{ij}) between an inidividual i and an individual j is defined as:

$$p_{ij} = 1 - \frac{|z_i - z_j|}{z_{max}} \tag{A.3}$$

where z_i and z_j are the phenotypic values of individuals i and j, respectively; and z_{max} is the maximum value of the phenotype distribution Z of the whole metacommunity. Thus, each pair-wise comparison, $p_{ij} \in \{0, 1\}$, is an element of the phenotypic similarity matrix P.

Evolutionary convergence and complementarity

We define evolutionary convergence as the similarity between average species phenotypes from distantly related species. We assume that two species are distantly related, in phylogenetic terms, if they do not come from a direct common ancestor, i.e. they are not sister species. To exclude sister species from the analysis we need to calculate the average genetic similarity among species of the same guild. The average genetic similarity between a species k and a species l is:

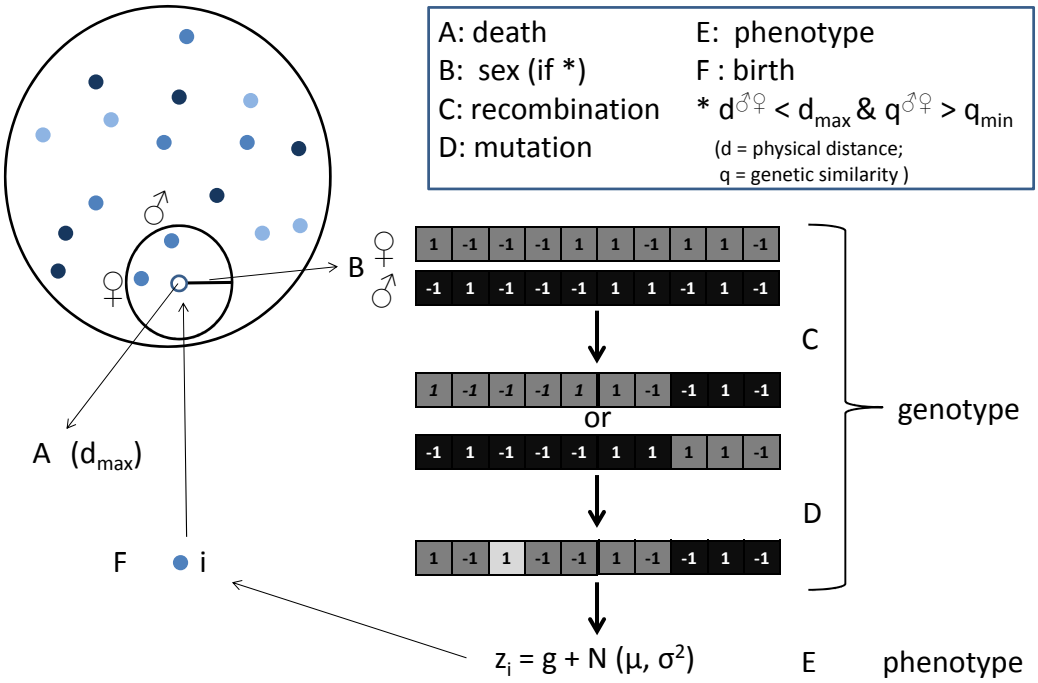


Figure B.1: General description of the model. Each time step in this model is completed after a death-birth cycle (from A to F). Individuals are represented as filled circles scattered across space and the variation of blue colors represents their variation of phenotypes. The model is divided into different events at each time step: (A) an individual k is randomly selected to die and leaves an empty location in the landscape. (B) a female individual i is randomly selected if $d_{kf} < d_{max}$ and this female f will mate and reproduce with a male individual j if conditions of mating are met ($d_{ij} < d_{max}$ and $q_{fj} > q_{min}$) (additional mating conditions depend on the guild, but always required the presence of a mutualistic partner, see Methods section). (C) The recombination process. Genomes are composed of L loci where each locus can be in two allelic states $(-1, 1)$ and undergo block cross-over recombination of the female genome (dark gray) and male genome (black), where a position l in the genome of the parents is randomly chosen partitioning the genome of each individual in two blocks. In this example the genome it is split into parts of equal length. All genes beyond the l locus in either organism's genome is swapped between the two parents and two new genomes are formed. (D) One of the two new genomes is randomly chosen for the offspring and it might undergo mutation (light gray). (E) The phenotype expression of newborn individual i is $z_i = g_i + \epsilon$. (F) The newborn i will occupy the site of the dead individual k within the area d_{max}.

Table B.1: Glossary of mathematical notation

Notation	*Definition*
d_{ij}	Geographical distance between individual i and j of the same guild
d_{max}	Maximum geographical distance to find a mating partner and dispersal
D	Geographic distance matrix containing all the d_{ij} values for a guild
d_{ik}^{PA}	Geographical distance between plant individual i and animal k
d_{max}^{PA}	Maximum geographical distance to find a mutualistic partner
D_{PA}	Geographic distance matrix containing all the d_{ik}^{PA} values
q_{ij}	Genetic similarity between individual i and j
Q	Genetic similarity matrix containing all the pairwise similarity q_{ij} values
q_{min}	Minimum genetic similarity above which i and j belong to the same species
p_{ij}	Phenotypic similarity between individual i and j
P	Phenotypic similarity matrix containing all the p_{ij} values
μ	Mutation rate per locus
N_e	Effective population size
z_i	Quantitative trait value of individual i
L	Size of the genome
ϵ	Environmental effect sampled from a Normal distribution of $\mu_\epsilon = 0$ and $\sigma^2 = 1$
g_i	Genetic effect of an individual i calculated as the sum of alleles across the genome
$\hat{p}_{kh}$	Average phenotypic similarity between species k and h
P_s	Phenotypic similarity matrix containing all $\hat{p}_{kl}$ values
P^{PA}	Phenotypic similarity matrix between plant and animal species
$\hat{q}_{kh}$	Average genetic similarity between species k and h
Q_s	Genetic similarity matrix containing all $\hat{q}_{kl}$ values

$$\hat{q}_{kl} = \frac{1}{n_k n_l} \sum_{i=1}^{n_k} \sum_{j=i}^{n_l} q_{ij} \tag{A.4}$$

where q_{ij} is the genetic similarity between an individual i of species k and an individual j of species l, and n_k and n_l are the absolute abundances of species k and l, respectively. The elements $\hat{q}_{kl}$ will form the matrix $Q_s \in \{\hat{q}_{kl}\}$ from which the sister species of each species in the guild can be identified.

To calculate evolutionary convergence we need to know the average phenotypic similarity between two species. We define phenotypic similarity between species k and l as:

$$\hat{p}_{kl} = \frac{1}{n_k n_l} \sum_{i=1}^{n_k} \sum_{j=i}^{n_l} p_{ij} \tag{A.5}$$

.

which is analogous to the definition of eq. A.4. This will build a species phenotypic matrix $P_s \in \{\hat{p}_{kl}\}$.

We then focus on each species in turn and exclude its sister species to avoid cases of parallel evolution to calculate the number of convergences related to the focal species. We

define a focal species k and a non-sister l species to be convergent if phenotypic similarity between them is higher than between focal and sister species ($\hat{p}_{k,sister} < \hat{p}_{kl}$) and higher than 0.95 ($\hat{p}_{kl} > 0.95$). A simple example to understand the calculation of convergences is illustrated in Figure B.2. With only three species, only one convergence is possible after excluding the sister species. Naturally, the number of convergences potentially increases with the number of species present. For example, if we have ten species and we exclude one of them as sister species, we have nine species to calculate convergence with. If we find that two out of nine species are phenotypicallly similar enough to the focal species, we count two (out of nine, ~ 22%) convergences. Thus, contrary to Guimaraes et al. (2011) we use both genetic divergence and phylogenetic relatedness for the estimation of evolutionary convergences, in order to avoid cases of parallel evolution [2] (Losos, 2011).

Evolutionary complementarity is easier to calculate because it does not involve the calculation of the genetic similarity matrix. We only need to estimate the phenotypic similarity between plant and animal species. We do this in the same way as for evolutionary convergence: we calculate the phenotypic similarity matrix $P_{PA} \in \{\hat{p}_{kh}\}$ between plant and animal average species traits and the condition for complementarity is that the similarity between a plant species k and an animal species h should be $\hat{p}_{kh} > 0.95$. To visualize the genetic relatedness between species we constructed clustering trees using Euclidean distance with the Python library ETE 2.01 (Huerta-Cepas et al., 2010).

Plant-animal interactions

In addition to the genetic and geographic constraints for mating, we consider other mating conditions that are different for each guild. These conditions describe the mutualistic interaction between plants and animals and their spatial constraints for interaction. We therefore specify another geographic distance matrix D^{PA} to describe the geographic distance between plant and animal individuals. Plant-animal mutualistic interactions are here described as follows: plants benefit from the presence of specific pollinators that are able to pollinate them and animals benefit from the presence of plants that provide resources for them. Thus, we have two extra conditions for mating: 1) Plants need the presence of animal pollinators within a close distance ($d^{PA}_{ik} < d_{max}$) and with a larger or equally-sized proboscis than the corolla of a plant: $z_c \leqslant z_p$. This corresponds to a physical or morphological constraint for individual interactions observed between plant and pollinator species (Stang et al., 2009, 2006). 2) Animals need the presence of plants within a close geographic distance ($d^{PA}_{jk} < d_{max}$). The conditions are illustrated in Figure B.1.

Our model allows bookkeeping of who is interacting with whom, i.e. this means we can record exactly which plant and animal individuals are interacting. This bookkeeping not allows comparison with high-resolution data of interactions, as in some plant-pollinator studies (Gómez et al., 2011; Gómez and Perfectti, 2012), but, more importantly for our current aim, enables us to identify the different constraints on the evolution and final topology of

2 Parallel evolution is the development of a similar trait in related, but distinct, species descending from the same ancestor

Q_s matrix

	a	b	c
a		0.85	0.97
b			0.89
c			

P_s matrix

	a	b	c
a		0.98	0.90
b			0.92
c			

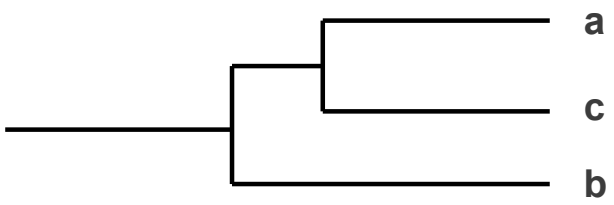


Figure B.2: Evolutionary convergence calculations. Convergence is calculated with the species genetic similarity matrix $Q_S \in \{\hat{q}_{kl}\}$ and the species phenotypic similarity matrix $P_S \in \{\hat{p}_{kh}\}$. This figure illustrates a simple example of evolutionary convergence where there are only three species in a guild (a, b and c). The upper matrix (Q_S) shows species a and c are genetically closely related, $\hat{q}_{ac} = 0.97$, while genetically distant from species b ($\hat{q}_{ab} = 0.85$, $\hat{q}_{cb} = 0.89$). A clear description of these genetic relationships can be represented with a cluster tree or dendrogram, as shown in the lower part of the figure. Thus, we establish that species a and c are sister species. The species phenotypic similarity matrix P_S shows that species a and b are phenotypically highly similar ($\hat{p}_{ab} = 0.98$) and highly genetically dissimilar ($\hat{q}_{ab} = 0.85$) (i.e. more than the average intraspecific genetic similarity or sister species 0.97), indicating an event of evolutionary convergence.

the network. We record the identity of the mutualistic partners during the reproduction process for plants and animals after reaching the steady-state to reconstruct the plant-animal interaction network.

Network topology

We measured three topological properties of plant-animal mutualistic networks: nestedness, connectance and centrality. The topological measurements were applied to the networks at the final steady-state of the simulation.

Nestedness

Nestedness describes a non-random pattern of species interactions where specialist species interact with proper subsets of more generalist species (Bascompte et al., 2003). We estimated nestedness using the NODF algorithm developed by (Almeida-Neto et al., 2008) because of its statistical robustness. NODF is based on standardized differences in row and column fills and paired matching of occurrences.

Connectance

Connectance measures the proportion of realized interactions (i.e. links) among all possible interactions in a network and is defined as $C = \frac{k}{P*A}$, where k represents the number of realized interactions between plant and animal species and P and A represent the number of plant and animal species, respectively, in the network (Jordano et al., 2003).

Centrality and node redundancy

To explore the topology of the network at the individual level we calculated three different centrality metrics: *degree centrality* (DC), *closeness centrality* (CC) and *betweenness centrality* (BC). These topological metrics are commonly used in social network analysis to study the importance of some nodes in the network, e.g. for the flow of information (Borgatti, 2005) or spread of diseases (Klovdahl, 1985). In ecological networks they have been used to describe the topology of individual-based interaction networks (Gómez and Perfectti, 2012; Gómez et al., 2011) and to identify keystone species that maintain the cohesiveness of the network (Jordán et al., 2007; González et al., 2010).

Degree centrality (DC) is defined as the fraction of nodes connected to a specific node(Borgatti and Halgin, ress). This metric provides a description of how well connected the individuals are. Closeness centrality (CC) measures the distance of a node to all the other nodes in the network; a node with high CC can potentially interact with any other node in the network (Borgatti and Halgin, ress; Newman, 2003). Betweenness centrality is the number of shortest paths between two nodes that pass through a specific node (Borgatti and Halgin, ress; Goh et al., 2003). Therefore, individuals (nodes) with high BC act as bridges, connecting one part of a network to another, maintaining the cohesiveness of the network. We also measured the average clustering coefficient (ACC) and node redundancy (NR) as complementary metrics of centrality. The average clustering coefficient computes the average probability, for any given node chosen at random, that two neighbors of this node are linked together (Latapy et al., 2008), hence it provides evidence of modularity in the network (Olesen et al., 2007). Node redundancy is defined as the fraction of pairs of neighbors of a specific node that are both linked to other nodes ($NR \in \{0, 1\}$). Therefore,

higher levels of node redundancy indicate that most nodes in the network share similar partner individuals and hence the elimination of those nodes from the network will not greatly affect the topology. All centrality and node redundancy computations were performed using the Python library NetworkX (Hagberg et al., 2008).

Simulations

We simulated a population size of $J = 10^3$ individuals for each guild and a genome size $L = 150$ loci. Larger population sizes ($10^4, 10^5$) are possible, but they are constrained by computational time. Initially all animal individuals have a higher phenotypic trait value than plant individuals ($Z_c \leqslant Z_p$) to assure that plant mating conditions are met at the beginning of the simulation. Geographic distance between each pair of individuals i and j, d_{ij}, was calculated as follows: 1) Euclidean coordinates of a two-dimensional space (x_i, y_i) were sampled from a uniform distribution ($x_i = [0, 1], y_i = [0, 1]$) for each individual in the metacommunity. 2) Using these coordinates (x_i, y_i) we calculated a matrix of relative Euclidean distances between the individuals (d_{ij}). This procedure is repeated for each of the geographic distance matrices (D_{PA}, D_{PP}, D_{AA}). The simulation lasted for 2×10^3 generations, where a generation is an update of J time steps. Steady-state was verified by checking the constancy of speciation events during the last 100 generations. We explored a range of parameter combinations with mutation rate, $\mu \in \{10^{-4}, 10^{-2}\}$, minimum genetic similarity $q_{min} = 0.97$ and maximal distance $d_{max} = 0.3$. We implemented the model in Python (and tested in IPython (Pérez and Granger, 2007)) and graphics were produced using the Python library Matplotlib (Hunter, 2007).

RESULTS

Mutation rates have an strong effect on the diversity dynamics by affecting the two types of speciation in this model: "mutation-induced" and "fission-induced" speciation. For large mutation rate ($\mu > 10^{-2}$), speciation is predominantly mutation-induced, resulting in the formation of a species consisting of a single individual. Most of these mutation-induced species are likely to go extinct because of their low initial abundance. For lower mutation rates ($\mu \in \{10^{-4}, 5 \times 10^{-3}\}$) speciation was predominantly "fission-induced", i.e. by slow genetic divergence between individuals, which also agrees with previous findings (Melián et al., 2012). Figure B.3 shows the mean incipient species size distribution for three different mutation rates. Low mutation rates produce low richness with few highly abundant species, i.e. highly positively skewed abundance distribution. Higher mutation rates ($\mu = 5 \times 10^{-3}$) tend to generate higher richness and less skewed species abundance distributions. The formation of species always follows the condition $q_{min} > Q*$, where $Q*$ is the mean genetic similarity of the matrix Q at equilibrium, as expected from analytical results of Melián et al. (2012). The consideration of plant-animal interactions and morphological constraints for plant reproduction, does not produce qualitatively different results in terms of species abundance distributions compared to previous models (Melián et al., 2012), that did not consider other mating constraints.

Table B.2: Predictions of the model and observed values in real mutualistic webs. Overall, qualitative predictions are very similar to observed ecological and evolutionary patterns. However, quantitatively we find many differences in the network topology, trait distribution and evolutionary patterns.

	Model predictions	*Real webs*
Nestedness	Highly nested	Highly nested (Bascompte et al., 2003)
Connectance	Medium connectance (0.5)	Low to medium connectance (0.01-0.38) (Dupont et al., 2009; **?**)
Centrality	Plants medium centrality and animals very low centrality	High centrality (Gómez and Perfectti, 2012)
Trait distribution	Bimodal or multimodal trait distribution	Log-normal distribution (right-skewed) (Stang et al., 2009)
Convergence	Low to medium convergence (17 %)	High levels of convergence (Bascompte and Jordano, 2007b)
Complementarity	Low to medium complementarity (20 %)	High levels of complementarity (Rezende et al., 2007a)
Diversity	Highly diverse (using low population size ~1000 indiv)	Highly diverse (Bronstein et al., 2006)

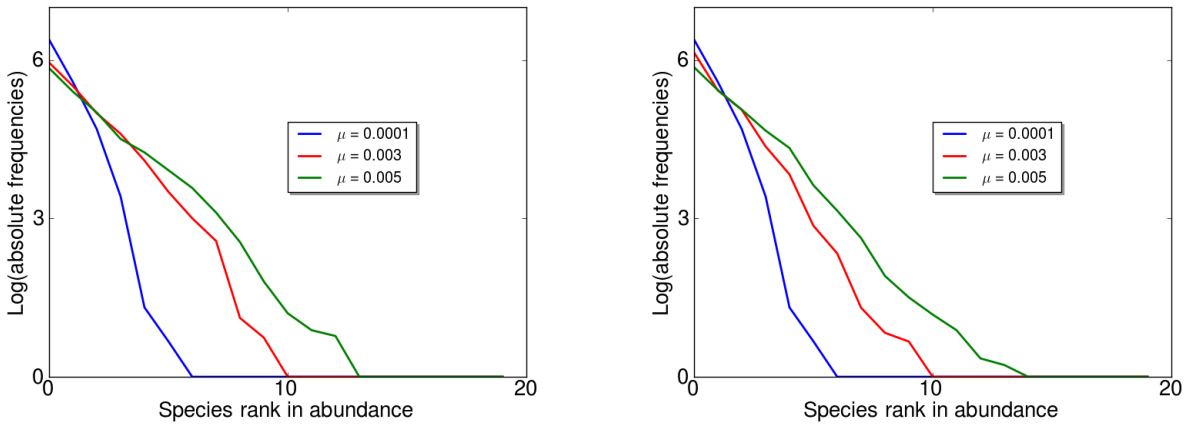


Figure B.3: Mean rank abundance distribution of animals (a) and plants (b) after 500 replicates for different mutation rates: $\mu = 5 \times 10^{-3}$ (green solid line), $\mu = 3 \times 10^{-3}$(red solid line) and $\mu = 10^{-4}$ (blue solid line). Parameters used: $q_{min} = 0.97$, $d_{max} = d^{PA}_{max} = 0.3$ and $J_P = J_A = 10^3$.

Genotype-phenotype relationship

The genotype-phenotype (G-P) relationship is highly positive as expected from the equation $z = g + \epsilon$. Figure A.1 shows a scatter plot of the G-P relationship of all pairs of individuals which contains three main clouds of points: 1) pairs of individuals of the same species with high genetic ($q_{ij} > q_{min}$) and phenotypic ($p_{ij} > 0.9$) similarity, 2) pairs of individuals of the same species with genetic similarity below q_{min} ($q_{ij} < q_{min} = 0.97$) and high phenotypic similarity ($p_{ij} > 0.9$) - these are incompatible individuals for mating and yet high phenotypic similarity $p_{ij} > 0.9$ and 3) highly genetically dissimilar individuals from different species ($q_{ij} \ll q_{min}$), but with the presence of highly phenotypically similar individuals ($p_{ij} > 0.9$). This is an indication of evolutionary convergence in plants and animals. An increase in mutation rate increases the genetic divergence between species, as expected, but it does not change the G-P relationship qualitatively (see Figure A.1). Naturally, we also find pairs of individuals with low genetic ($q_{ij} \ll q_{min}$) and phenotypic ($p_{ij} < 0.9$) similarity.

Evolutionary convergence and complementarity

The plant and animal trait distributions (i.e. corolla and proboscis lengths) change dramatically during the simulation because of the speciation process, which generates changes in the trait distribution on both guilds resulting in a bimodal distribution (Figure B.4). Figure B.4 also shows an example of the variation of traits at the species level, where several species of the same guild (plant or animal) can have highly similar trait values (i.e. evolutionary convergence). The figure also shows that there are similar trait values between plant and animal species (i.e. evolutionary complementarity).

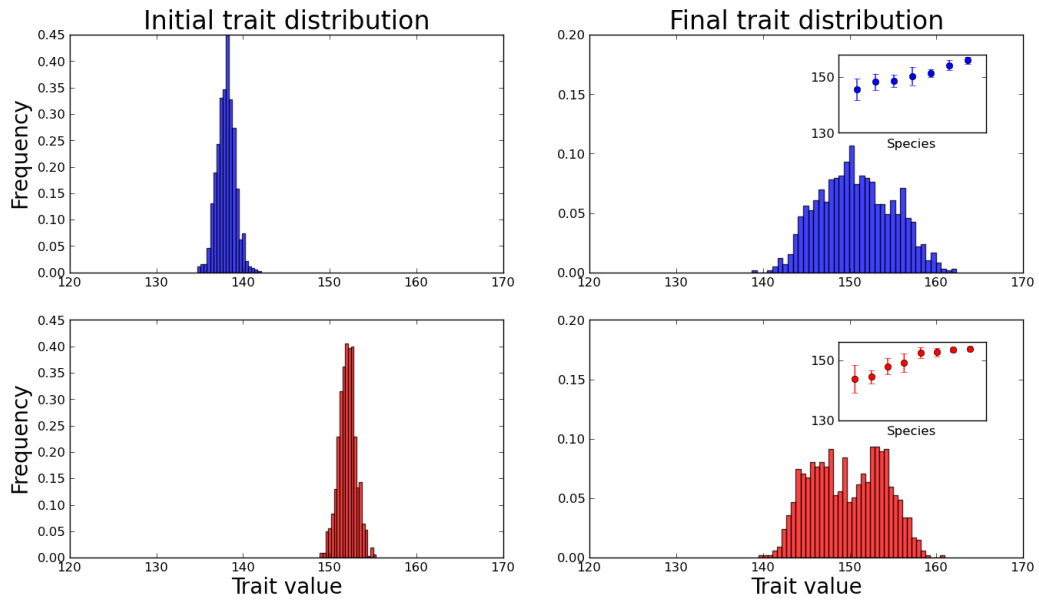


Figure B.4: Changes in trait (i.e. phenotype) distribution of plants and animals for a typical replicate simulation. The top panels show the changes in animal (red) trait distribution and bottom panels the changes in plant (blue) trait distribution. Left panels show the initial trait distribution and right panels the final trait distribution. The insets in the right panels show the mean and standard error deviations of traits. The trait distribution changes completely from the initial distribution towards a bimodal distribution in both guilds. Parameters used: $q_{min} = 0.97$, $d_{max} = d_{max}^{PA} = 0.3$, $\mu = 5 \times 10^{-3}$ and $J_P = J_A = 10^3$.

Evolution of convergence and complementarity occurs in all replicate simulations. Evolutionary convergence appears on average in $17.3 \pm 6\%$ of all possible cases. The number of convergences mainly depends on the number of sister species pairs, i.e. an increase of the number of sister species pairs will decrease the possible number of convergences. Evolutionary complementarity appears with a similar frequency in each replicate simulation, but with a larger variation ($20 \pm 18\%$) than convergence. Complementarity mainly depends on the number of plant and animal species; therefore the variation in the number of species at the steady-state between guilds affects the number of complementarity events. An example of evolutionary convergence and complementarity of one replicate is shown in Figure B.5.

Network topology

The network at the species level is highly nested $\overline{N} = 69.97 \pm 13.4$ as in real plant-pollinator networks and medium level connectance $\overline{C} = 0.5 \pm 0.07$ (Figure B.6). At the individual level, there is high centrality for a low number of individuals in the whole network regardless of species differences. High centrality also occurs at the intraspecific level. Most individuals in the network have low degree centrality ($DC \leqslant 0.01$) and only few individuals have

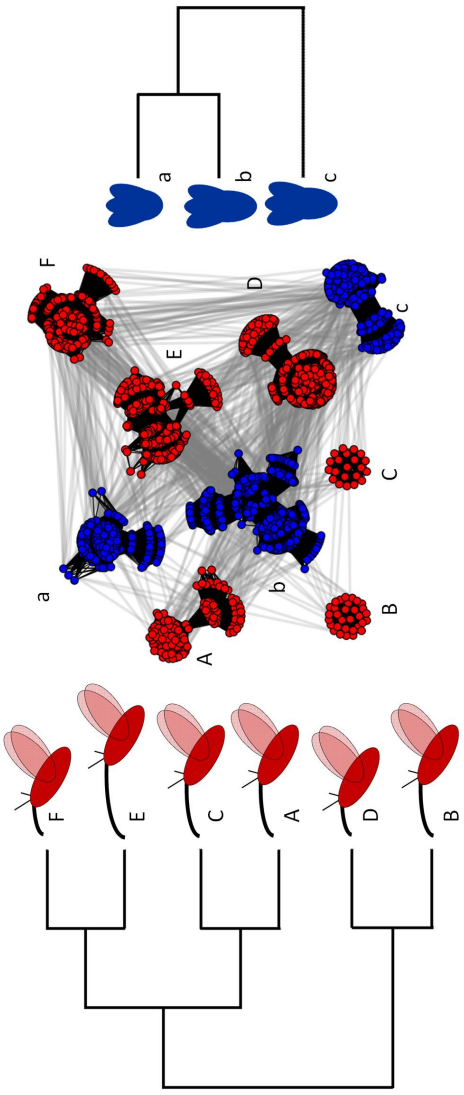


Figure B.5: Evolutionary convergence and complementarity in plant-pollinator networks. Cluster trees at the top and the bottom, show genetic similarities between plant (blue) and animal (red) species, respectively. The average species trait, proboscis and corolla length, is sketched with cartoons next to their respective position in the cluster trees. Animals, composed of six species, have two convergent trait events (species A-B, A-C and F-D), while plants, composed of three species, only have one convergent event (species b-c). The central figure shows the network of plant-animal interactions, where each node (colored filled circles) represents an individual in the metacommunity. The network is composed of two types of links: genetic relatedness links (black solid lines) forming clusters that represent species and plant-animal individual-based interaction links (gray lines). The network shows variability in terms of genetic relatedness and plant-animal interactions within a species (i.e. high intraspecific variability). This figure is an example from one replicate simulation. Parameters used: $q_{min} = 0.97$, $d_{max} = d_{max}^{PA} = 0.3$, $\mu = 5 \times 10^{-3}$ and $J_P = J_A = 10^3$.

high DC ($DC \gg 0.01 \sim 100$ links), which is shown by the positive skewness of the DC distribution (see table A.1) and the intraspecific variation (see Figure A.2). The betweenness centrality (BC) distribution follows a similar pattern of high intraspecific variation, but less interspecific variation (see Figure A.2). The BC distribution is more positively skewed than the DC distribution (see table A.1) because of the presence of few individuals with very high BC ($BC > 0.04$), this is more evident in plants than animals. Therefore, the distribution of DC and BC shows that only very few individuals serve as connectors or bridges between species of the opposite guild . High average BC is positively correlated with species abundance ($R^2 = 0.798, p < 0.001$) and partner diversity (i.e. number of partner species) ($R^2 = 0.54, p < 0.001$). Also, there is a high correlation between centrality metrics for both plants and animals (see Table A.2). This clearly indicates the importance of these individuals for maintaining the cohesiveness in the network. Although, plants and animals only show slight differences in terms of centrality (see table A.1), plant centrality metrics distributions are more asymmetric. The higher asymmetry might be caused by the morphological constraint on plant reproduction. Most individuals have medium levels of closeness centrality ($\overline{CC} = 0.45$). Interestingly, most individuals have neighboring peers in the network that are also interacting with the same mutualistic partners, as indicated by the high level of node redundancy ($NR > 0.5$). However, a few individuals, especially those with high BC, have low node redundancy ($NR < 0.3$) and their extinction could greatly affect network topology. Only the correlation between NR and DC is significantly positive for plants, but not for animals (see Table A.2). The average clustering coefficient is higher in plants ($ACC = 0.053$) than animals ($ACC = 0.038$), which suggests that plant network topology tend to be more modular or compartmentalized than animal network topology.

DISCUSSION

Considering main evolutionary forces in the study of community assembly is crucial to understand the emergence of observed ecological and evolutionary patterns. Several theoretical studies have investigated the evolution of ecological communities assuming niche-related processes as the main drivers of community structure and diversity (Caldarelli et al., 1998; Loeuille and Loreau, 2005; Ingram et al., 2009). Nevertheless, models that only consider neutral processes (e.g. dispersal limitation, ecological drift) are also able to reproduce observed patterns of community diversity and structure (Rosindell et al., 2011). A controversial point of neutral theory was the unrealistic assumption of point-mutation speciation (Hubbell, 2001). This point has been later improved in other models by considering a gradual speciation process phenomenologically (i.e. protracted speciation (Rosindell et al., 2010), see also **?**) and mechanistically (i.e. by modelling genetics explicitly (Melián et al., 2012, 2010; de Aguiar et al., 2009)). Genetically and spatially explicit neutral models allow a connection between genetics and community ecology. Our model makes this connection by considering an explicit speciation process and its consequences for the diversity and structure of mutualistic networks. Furthermore, this is the first model, to our knowledge, to study the joint evolution of network structure and quantitative traits in mutualistic networks. Our results show the emergence of some observed topological properties of mutualistic webs and the evolution of trait convergence and complementarity (see Table **??**).

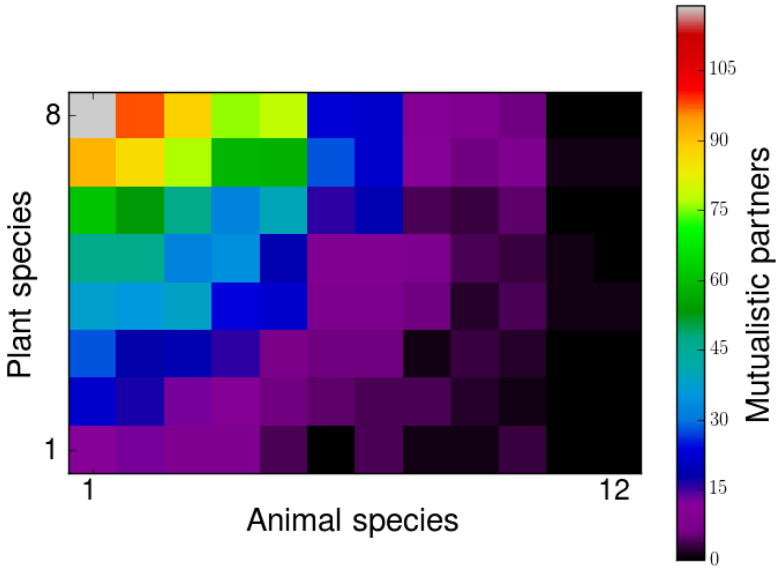


Figure B.6: Plant-animal species interaction network. Plant species are represented in rows and animal species in columns. The color gradient indicates the number of mutualistic partners (i.e. individuals interacting) shared between plant and animal species. This matrix comes from one replicate with nine plant and twenty animal species. The network shows high level of nestedness ($N = 0.72$) and intermediate level of connectance ($C = 0.5$). Parameters used: $q_{min} = 0.97$, $d_{max} = d^{PA}_{max} = 0.3$, $\mu = 5 \times 10^{-3}$ and $J_P = J_A = 10^3$.

Similar to previous neutral genetically explicit eco-evolutionary models (Melián et al., 2012; de Aguiar et al., 2009), two important factors of the speciation process are non-random mating (q_{min}) and dispersal limitation (d_{max}). These two factors determine the diversity of communities. Interestingly, Kondrashov and Shpak (1998) found that assortative mating alone in the absence of selection is sufficient to create genetic divergence between individuals and finally the formation of species. Naturally, quantitative changes in the three evolutionary forces considered here (mutation, recombination, genetic drift) are main drivers of the genetic variation in our model and it will ultimately affect diversity patterns. Mutation rate alone can already change the speciation dynamics and species abundance distribution as also shown by Melián et al. (2012). However, dispersal limitation is also a very important driver of the speciation dynamics. It basically determines the gene flow in the metacommunity (**?**) and therefore can help to reinforce the speciation process together with non-random mating (**?**). Thus, high assortative mating (high q_{min}) and high dispersal limitation (low d_{max}) can maximize the diversity (Melián et al., 2012; de Aguiar et al., 2009).

Convergence, complementarity and drift

Evolutionary convergence, i.e. the independent evolution of similar features in different evolutionary lineages, of traits is observed in all our replicate simulations and with little variation. Evolutionary convergence has been argued to be a product of multispecific coevolutionary processes ('diffuse coevolution')(Janzen, 1980; Jordano et al., 2003; Bascompte and Jordano, 2007b; Thompson and Cunningham, 2002) and therefore these patterns are molded by similar selective pressures; as shown by Guimaraes et al. (2011). However, our model shows that evolutionary convergence can occur through the action of the non-selective forces of mutation, recombination and genetic drift. This means that random evolutionary change can cause species to become more similar to each other than their ancestors were , as also shown by Stayton (2008). Stayton (2008) simulated evolution along phylogenies according to a Brownian motion model of trait change and demonstrated that rates of convergence can be quite high when clades are diversifying under only the influence of genetic drift. Furthermore, constraints (e.g. developmental constraints) in the production of variation can also lead to convergence. For example, if the variation produced is limited, then unrelated species are likely to produce the same variation, which may then become fixed in the population by genetic drift (Losos, 2011; Stayton, 2008). This a common feature of biological systems because in the evolution of DNA there are only four possible states for a given nucleotide position, and therefore it is likely that distantly related taxa will independently acquire the same change by chance (Losos, 2011). It can also happen in our model because there are many genotypes that can give the same phenotype.

Developmental constraints are a common explanation for the convergence of traits (Solé et al., 2002b; Losos, 2011). However, we still know little about how developmental constraints affect convergence. The tinkering of traits by evolutionary forces largely affect developmental pathways (e.g. gene regulatory networks) (Solé et al., 2002b). Thus, developmental pathways are not static, but can diverge through time randomly without substantially affecting phenotype. This is called developmental system drift (DSD) (True and Haag, 2001). We argue that DSD might play an important role in the evolution of morphological traits and it must be considered as another level where drift can be acting (Ohta, 2002), for example, by considering random wiring in gene regulatory networks.

Evolutionary complementarity is also consistently observed in our results but with a larger variation than convergence. Complementarity is argued to be the main result of tight coevolution between mutualistic species by mechanisms, such as trait-matching (e.g. corolla length-proboscis length) (Jordano et al., 2003). There is empirical (**?**) and theoretical evidence (Gomulkiewicz et al., 2000) for coevolutionary hot spots (Thompson, 1999), which suggests that local selective regimes can promote the coevolution of traits (Ferdy et al., 2002; Bronstein et al., 2006; Gomulkiewicz et al., 2000, 2003; Jones and Ferrière, 2009; Jordano et al., 2003; Thompson, 2009; Thompson and Cunningham, 2002). However, we show that low to medium levels of complementarity and convergence can be the product of neutral processes occurring at several levels (i.e. genome, development).

Evolution of quantitative trait distribution

Our model predicts that the distribution of traits, regardless of species differences, generally evolves towards a bimodal distribution of phenotypes. This result was previously obtained by Kondrashov and Shpak (1998), who assumed absence of selection and assortative mating in a infinite population. Their results show the evolution of traits into two phenotypic classes. Strong assortative mating produces high correlations of allelic effects among all loci, which leads to the evolution of two phenotypic classes: one with alleles increasing the trait and the other with alleles decreasing the trait (**?**). Devaux and Lande (2008) found similar results using a finite diploid population with multiple alleles per locus and they showed that the splitting of the phenotype distribution is possible under strong assortative mating and genetic drift, but the distribution is transient rather than permanent. However, our distribution is not transient, and this is probably because we only considered two allelic states for each locus. As Devaux and Lande (2008) explained, by assuming a normal distribution of allelic effects at each locus we could obtain a more continuous unimodal (i.e. normal) distribution of phenotypes. We need further analytical exploration to thoroughly understand the determinants of trait distributions.

We find a gradient of a species phenotypes from low to high average values (Figure B.4). Therefore, a whole spectrum of species phenotypes can emerge in the metacommunity by stochastic processes. However, the predicted trait distribution is not right-skewed as observed in real plant-pollinator communities (Stang et al., 2009) (see Table **??**). This might be due to the influence of other forbidden links (e.g. body size) and developmental constraints not considered in this model.

Neutrality in mutualistic networks: patterns and processes

The morphological constraint for plant reproduction does not seem to change the diversity patterns in plants compared to animals (Figure B.3). This suggests that considering this 'forbidden link' has no effect on the speciation dynamics of mutualistic networks. However, the topology of plants and animals seems to be slightly different in terms of centrality metrics (i.e. degree and betweenness centrality). Plant topology is more asymmetric than animal topology, which is probably due to the morphological constraint for plant reproduction. This supports the idea that size thresholds of plant and animal mutualistic traits and species abundances promote asymmetry in mutualistic networks (Stang et al., 2009, 2006). The presence of individuals with high centrality (BC and DC) in high-resolution plant-pollinator webs has been found to be related to the fitness of individuals and probably related to specific phenotypes (Gómez and Perfectti, 2012). In our results, high centrality in some plant individuals might be related to the morphological constraint in plant reproduction. Furthermore, higher average clustering coefficient seems higher in plants than animals and it is also probably related to the morphological constraint. This suggests that modularity observed in real mutualistic webs, here indicated by higher average clustering, can be partly an outcome of biological constraints (i.e. forbidden links) (Olesen et al., 2007).

Connectance values are close to the predictions of other neutral network models (Canard et al., 2012) with similar diversity values. However, compared to real mutualistic networks

with similar diversity as ours (24 plant and animal species on average), our connectance ($\overline{C} = 0.5$) is higher than reported webs ($C = 0.28$) (**?**). This difference in connectance values might also be due to other forbidden links, such as phenology (Olesen et al., 2010).

Nestedness values are also very high, as in real mutualistic networks. The influence of stochastic eco-evolutionary processes and the morphological constraint seems to predict realistic values. However, we think that stochastic processes are more important in determining nestedness. This is based on previous neutral models (Krishna et al., 2008; Canard et al., 2012), which suggests that random interactions, dispersal limitation and species abundance distribution ('neutral forbidden links' (Canard et al., 2012)), are determinants of the structure of mutualistic networks.

Future directions

We have only explored a limited range of the parameter space. For example, we could still explore the effects of genetic similarity (q_{min}) and spatial structure (d_{max}, d^{PA}_{max}) on the diversity and structure of mutualistic webs. Based on previous models (Melián et al., 2012; de Aguiar et al., 2009) we expect changes in the diversity of the metacommunity. For example, high values of q_{min} and shorter geographical distances for mating (d_{max}) should generate a higher diversity in the metacommunity (Melián et al., 2012). However, extremely low geographic distances for mating could decrease the diversity due to the difficulty of finding mates (Allee effect), especially for high q_{min} levels.

In our model, assortative mating and the morphological trait are determined for the same multiple loci (i.e. they have the same genetic basis) and these genes show pleiotropic effects. Assortative mating and morphological traits are calculated in a similar way: the sum of genetic differences. This closed relationship between nonrandom mating and an ecological trait is similar to the concept of 'magic' traits. A 'magic' trait combines a trait subject to divergent selection and another trait related to nonrandom mating (i.e. reproductive isolation) that are pleiotropic expressions of the same gene(s) (Servedio et al., 2011). However, we cannot regard our trait as 'magic' because of the absence of disruptive selection forces. There are other alternatives for this relationship between assortative mating and the morphological trait (Servedio et al., 2011). One alternative is that assortative mating and the morphological trait are determined by different sets of genes and express different levels of pleiotropic effects (i.e. a partly 'magic' trait (van Doorn and Weissing, 2001)).

One might also explore further the influence of the morphological constraint in the evolution of traits. This constraint might be exerting a weak selection force on the evolution of plant traits in our model. The comparison with other models without any morphological constraint (i.e. only non-random mating) and with morphological constraints for animals and plant reproduction (i.e. phenotypic match), might elucidate the importance of morphological constraints in the evolution of the network.

The possibility to test model predictions with high-resolution data is one of the most important advantages of our model. Plant and pollinator species abundance data, intraspecific trait variation, genetic data, spatial distribution can all be used to test model predictions. Accounting for intraspecific variation helps explaining emergent properties of ecological networks and evolutionary patterns (Bolnick et al., 2011).

We conclude that simple processes (dispersal, demography, mutation, recombination and morphological constraints) can reproduce very well the observed network structure and quantitative trait evolutionary patterns in plant-animal mutualisms.

ACKNOWLEDGEMENTS

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APPENDIX: SUPPORTING FIGURES AND TABLES

Table A.1: Topology of individual-based plant-pollinator network. Node redundancy (NR) and three centrality metrics were calculated: degree centrality (DC), closeness centrality (CC) and betweenness centrality (BC). The estimates show the skewness, mean and standard error values of the calculated distributions for each metric. The calculations were made for each guild, plants and animals, considering all individuals regardless of species differences. Parameters used: $q_{min} = 0.97$, $d_{max} = d^{PA}_{max} = 0.3$ and $J_P = J_A = 10^3$.

	Plants		Animals	
	Mean±std	Skewness	Mean±std	Skewness
DC	0.01±0.012	1.87	0.01±0.013	2.08
CC	0.45 ± 0.045	-0.97	0.45 ± 0.044	0.11
BC	0.00058 ± 0.0013	4.47	0.00058 ± 0.0014	4.78
NR	0.56 ± 0.39	-0.37	0.53 ± 0.4	-0.38

Table A.2: Correlations between centrality metrics and between centrality and node redundancy. BC: Betweenness centrality, CC: closeness centrality, DC: degree centrality, NR: node redundancy. * (p<0.001). Most correlations between centrality metrics are significantly positive. However, correlations between node redundancy and centrality metrics was only significant in plants degree centrality (DC).

	BC	*CC*	*DC*	*NR*
BC		0.44*	0.93*	0.15
CC			0.5*	0.15
DC				0.41*
NR				

(a) Plants

	BC	*CC*	*DC*	*NR*
BC		0.35*	0.9*	0.08
CC			0.3*	0.01
DC				0.03
NR				

(b) Animals

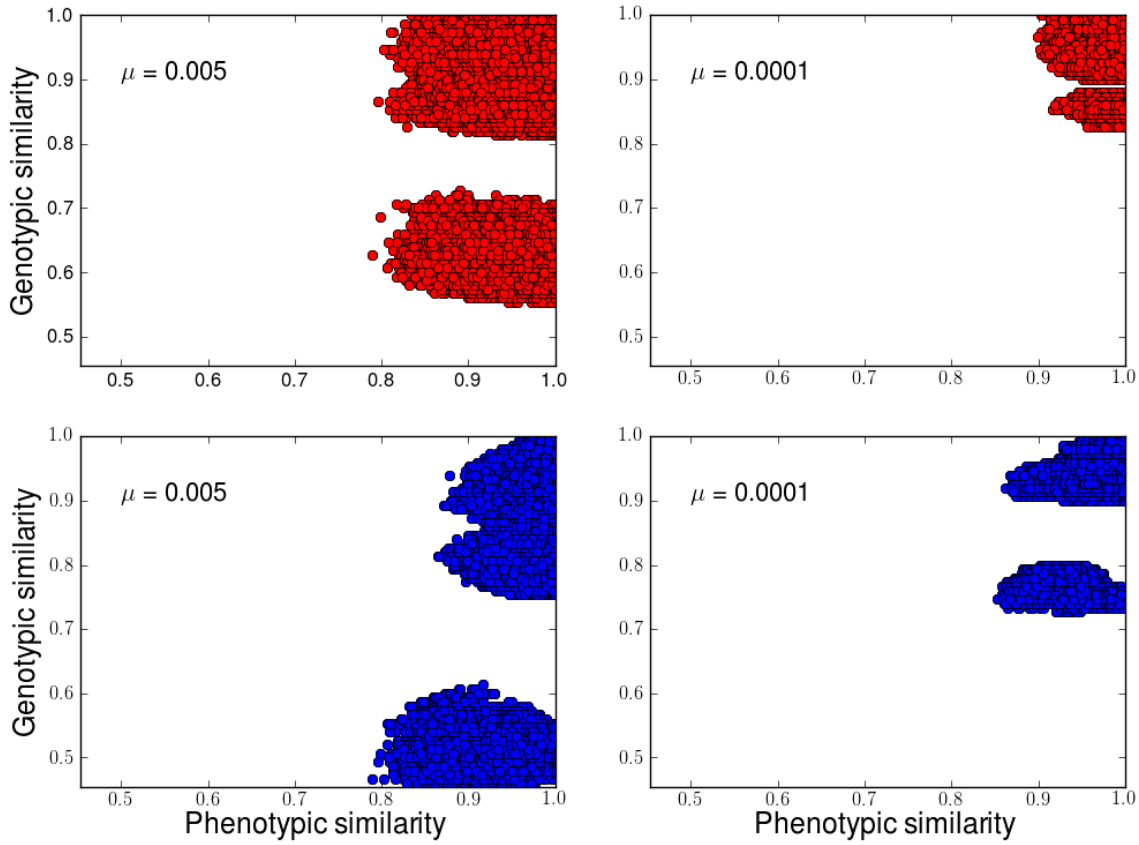

Figure A.1: The effect of mutation rate on the genotype-phenotype (G-P) relationship. Top panels show the G-P relationship for animals (red) and bottom panels for plants (blue). Right panels show the G-P relationship for mutation rate $\mu = 5 \times 10^{-3}$ and left panels for $\mu = 10^{-4}$. Each plot is an scatter plot, where each filled circle represents phenotypic ($p_{i \neq j}$, x_axis) and genetic ($q_{i \neq j}$, y-axis) similarity between two individuals of a particular guild (plant or animal) from one replicate. The G-P correlation can be very positive or close to zero depending on the individuals compared. Individuals with high phenotypic similarity and genetic dissimilarity suggests evolutionary convergence of traits, regardless of mutation rate. Parameters used: $q_{min} = 0.97$, $d_{max} = d^{PA}_{max} = 0.3$ and $J_P = J_A = 10^3$.

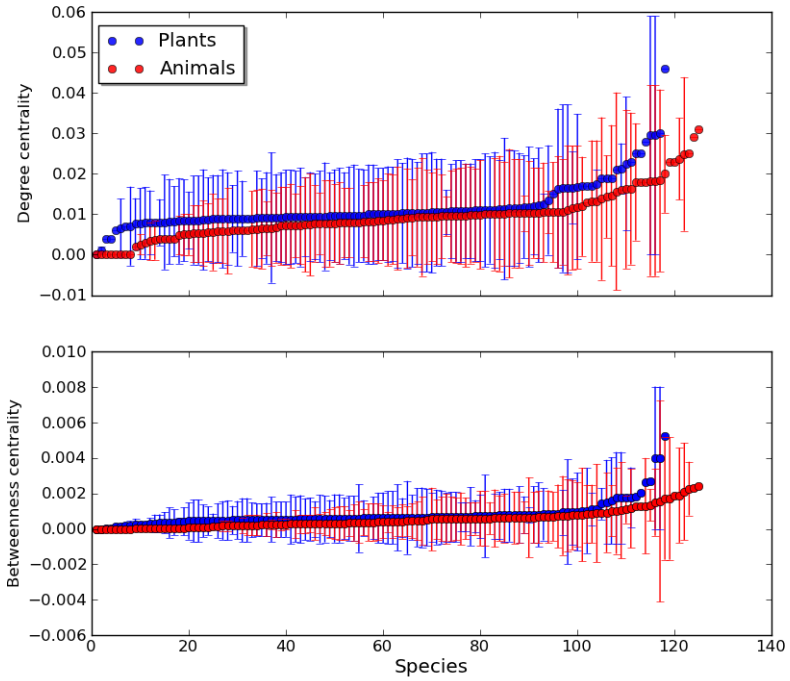

Figure A.2: Variation of centrality measurements between and within species. The top panel shows the variation of degree centrality (DC) and the bottom pannel shows the variation of betweenness centrality (BC). Each filled circle represents the average value of DC and BC for one species with their respective standard deviation (vertical thin lines) for plants (blue) and animals (red). The plots represent a sample of 50 replicates. The interspecific and intraspecific variation of BC and DC is quite heterogeneous; a few species tend to have higher BC (> 0.001) and DC (> 0.02) with a large intraspecific variation.

7

SYNTHESIS

> "*The animal species, in which individual struggle has been reduced to its narrowest limits, and the practice of mutual aid has attained the greatest development, are invariably the most numerous, the most prosperous, and the most open to further progress. The mutual protection which is obtained in this case, the possibility of attaining old age and of accumulating experience, the higher intellectual development, and the further growth of sociable habits, secure the maintenance of the species, its extension, and its further progressive evolution. The unsociable species, on the contrary, are doomed to decay*."
> Kropotkin (1955)

Positive feedbacks between species can occur in many ways, directly or indirectly (Stachowicz, 2001). The repercussions on the dynamics of communities can be manifold, from positive to negative (Loreau, 2010). Therefore, cooperation between species *per se* does not always imply a positive outcome of the parties involved. It might highly depend on the ecological context (local biotic and abiotic conditions) where species interact and the benefit-cost ratio (Holland and Bronstein, 2008; Holland, 2002; Holland et al., 2002). The inherent conflict of interests between species (or invidividuals) and the ecological situation render the final outcome of the interaction (Bronstein et al., 2006). This is not different from trading and financial transactions between agents looking to increase their own profit or success. Thus, selfish actions are always behind the interests of the individuals, a thought originating from Darwin (**?**).

The evolution of mutualism has always posed a problem to evolutionary biologists: why does an individual from one species provide benefits to another individual of another species? One common explanation is reciprocal altruism (Trivers, 1971), and this has usually been studied with the Prisoner's Dilemma (PD). If the same partners interact repeatedly (iterated PD), then the best strategy can be to cooperate with other individuals who also cooperate (Axelrod and Hamilton, 1981). However, in a multispecific and spatially structured scenario, such as metacommunities, how can mutualism be stable, and what is its role in the dynamics of metacommunities and ecosystems? Surprisingly, these are questions that still remain largely unanswered. The importance of mutualism on a broader level, at the ecosystem level, is still not fully understood. Olff et al. (2009) and Ulanowicz and Hannon (1987) argued that indirect mutualism is an organizational force in food webs, as the resulting feedback loops 'attract' resources towards them. Other studies suggest that mutualism can be a stabilizing force in communities under certain conditions (Bascompte et al., 2006; Bastolla et al., 2009).

The stability of mutualistic communities depends on several factors, probably common to all communities (e.g. spatial structure (Holt, 2002), environmental variability (Chesson, 1986)), and others specific to the life-history of the species involved (Holland and Bronstein, 2008). In the following sections I will discuss the main results and future directions of this thesis. I will start discussing how the ecological and evolutionary stability of mutualistic systems is affected by their life-history and other ecological interactions. Then, I will explain the importance of spatio-temporal variability to understand their dynamics and explain main topological properties of plant-animal mutualistic networks. Finally, I will talk about future directions in the development of eco-evolutionary dynamics models of mutualistic webs and the test of models with empirical data.

7.1 THE STABILITY OF MUTUALISTIC SYSTEMS

Classical theoretical studies of mutualism explored their stability conditions. May (1976) found that mutualism is inherently unstable. Later, Vandermeer and Boucher (1978) and others (Wolin and Lawlor, 1984; Soberon and Martínez del Río, 1981; Addicott, 1981; Dean, 1983) found that mutualism can be stable if the benefits are finite and intraspecific density-dependence is taken into account. This makes the system stable under different conditions mainly depending on whether interacting species are obligatory or facultative mutualists (Vandermeer and Boucher, 1978). If mutualism is obligatory for one or both partners there is a positive density-dependence effect (i.e. Allee effect) that affects the stability conditions of the interaction (Wolin and Lawlor, 1984). This effect disappears if the mutualism is facultative (Dean, 1983). However, the interest in more mechanistic models and the consideration of life-history have changed the path of research in this area (Holland and DeAngelis, 2010; Holland et al., 2002, 2004a; Wilson et al., 2003). In this thesis, I have studied the importance of life-history on the stability of plant-animal mutualisms (Chapters 2 and 3).

7.1.1 *The importance of life-history*

Several studies on models of specific mutualistic systems have shown that considering the life-history of the species is important to predict the stability conditions of mutualisms (Wilson et al., 2003; Bronstein et al., 2003b; Holland, 2002; Holland et al., 2004a). In the fig-fig wasp system, the consideration of different life-stages (eggs, larvae, seeds, fruits) has been crucial to understand the dynamics of this complex interaction (Jones and Ferrière, 2009; Wilson et al., 2003; Ferriere et al., 2007). Surprisingly, in models of the population/community dynamics of more common plant-pollinator or plant-seed dispersal systems the consideration of life-history is scarce, even though most pollinators are insects with complex life cycles and their life-stages undergo different selective pressures and usually live in different environments (Knight et al., 2005). Thus, important biological details of common plant-animal mutualisms remain unexplored in theoretical studies. This is all the more surprising because population structure has been shown to be important in theoretical studies of predator-prey systems to largely influencing the stability of the interaction (Nisbet and Gurney, 1983) and in ecological networks (Rudolf and Lafferty, 2011). Complex ecological

feedbacks between life-stages and the species they are interacting with has been studied extensively in predator-prey systems (Roos et al., 2003b; Rudolf, 2007; De Roos et al., 2007).

Life-history has profound implications for the dynamics of plant-pollinator systems (Chapter 2). The population structure of pollinators (adult:larva ratio) is crucial for the plant to obtain an efficient pollination service and consequently for the pollinators to survive and coexist with the plants. Therefore, external factors impairing an specific life-stage will affect population structure and consequently the dynamics between plants and pollinators (Chapter 2). In real communities, pesticides are among the most important factors reducing larva maturation rate in insect pollinators (Krupke et al., 2012; Morandin and Winston, 2003; Wu et al., 2011), therefore affecting population structure. This has been mainly studied in Hymenopteran pollinators, such as the honey bee (Krupke et al., 2012). Decreasing pesticides effects in the environment will still have long-term effects on the sensitive life-stages and the population might never recover (Desneux et al., 2007). This also happens in other animals with complex life cycles, such as amphibians (Bridges and Semlitsch, 2000; Relyea et al., 2005). Furthermore, interactions between pesticides and pathogens can be a major contributor to increased mortality of honey bee colonies, including colony collapse disorder, and other pollinator declines worldwide (Pettis et al., 2012b).

7.1.2 *Mutualism and other interactions*

Most models of ecological networks are based on a single type of ecological interaction. These studies are very important as a first step to understand the dynamics of ecological networks. Nevertheless, they obviously do not represent the complexity found in real ecological communities and ecosystems. All these networks are intertwined interacting with each other simultaneously. Therefore, theoretical progress in ecological network studies is now starting to merge different ecological networks and study their dynamics. A straightforward case is to study networks with trophic and non-trophic interactions. For example, Melian et al. (2009) studied a herbivore-mutualistic network combining three different guilds: plants, herbivores and pollinators. Interestingly, they found that the balance between mutualism and antagonism and the distribution of interaction strengths significantly affected community diversity. An important step in future studies will be to integrate the ecosystem perspective into ecological network models. Some studies have already started this avenue of research where fluxes of energy and recycle of matter are considered in the dynamics (Goudard and Loreau, 2008; Olff et al., 2009).

The dark side of mutualism

Mutualism in a multiple interaction context does not necessarily have a positive effect on the community. Interestingly, mutualism can also be damaging for diversity when there is strong intraguild competition in the community. This is what I found studying mutualistic network dynamics with intraguild competition: communities with strong intraguild competition and mutualistic interactions generate low diversity compared to communities with weak intraguild competition. Moreover, with only intraguild competition we obtain a higher diversity than with both, mutualism and intraguild competition, because mutualism gives an advantage to species under strong competition, and hence mutualist species with strong

mutualistic interactions can out compete other species with weaker mutualistic interactions (Chapter 4).

Goudard and Loreau (2008) found very similar results studying an interaction web model with trophic and non-trophic (e.g. mutualism, facilitation) interactions. Interestingly, they found that non-trophic interactions tend to reduce biomass and ecosystem production at all trophic levels. The question is why? As Loreau (2010) says, the reason is paradoxical: networks with trophic and non-trophic interactions become more efficient in resource exploitation. Therefore, although positive interactions (i.e. mutualism and facilitation) can improve the use and transference of resources along the food chain, they can also aggravate the negative effects of trophic interactions when consumers are generalists, such as overexploitation and strong resource and apparent competition (Loreau, 2010). Thus, compared to a purely trophic network, non-trophic interactions could decrease diversity and ecosystem functioning (Goudard and Loreau, 2008). Actually, as Allesina and Tang (2012) found, purely trophic networks are more stable than mixed networks (non-trophic and trophic interactions). The explanation is the same in both models (Encinas-Viso et al., 2012; Goudard and Loreau, 2008): the benefits of positive interactions can feedback negatively in the whole network (or ecosystem) through more competition and/or exploitation reducing diversity.

An interesting question is what conditions allow the evolutionary emergence and stability of these mixture systems (mutualistic and trophic interactions)? Again, the answer seems related to the dynamical balance between negative and positive effects and this balance is probably shaped by the action of multiple levels of selection. The formation of multiple levels of selection seems to have shaped living systems on this planet (Hogeweg and Takeuchi, 2003), and more interestingly different models show that the emergence of new levels of selection is an inevitable property of eco-evolutionary processes when interactions occur in a spatially structured landscape (Boerlijst and Hogeweg, 1991; Hogeweg, 1994). Thus, ecological feedbacks between trophic and non-trophic interactions should be shaped by multiple levels of selection maintaining the balance and stability of the system. Interestingly, it is also very likely that these systems with positive interactions have several alternative stable states (Kéfi et al., 2008).

Mutualism and antagonism in plant-pollinator interactions

Antagonistic and mutualistic interactions often occur in plant-animal mutualistic communities (Fontaine et al., 2011; Bronstein et al., 2006). During the larval stage of many insect pollinators, such as Lepidopterans, the larvae consume plant tissue, i.e. they are herbivores (Adler and Bronstein, 2004). Larvae feed on plant leaves to mature and become adult pollinators. These ontogenetic diet shifts (Rudolf and Lafferty, 2011) are very common and important to understand the ecological and evolutionary dynamics of plant-animal mutualisms. Interestingly, in some cases the larvae feed on the same plant species that will be later pollinated by the adult pollinator (Adler and Bronstein, 2004). This shows that in several cases mutualistic and antagonistic interactions are exerted by the same species. Naturally, for this interaction to be stable mutualistic benefits should outweigh the damaging effect of the larval stage and this is what I found studying a plant-pollinator model with mutualism (pollination) and antagonism (herbivory) (see Figure A.1). A preliminary analysis of the

model shows that the interaction can be stable if mutualistic benefits for the plant outweigh the damage caused by herbivory; however, if herbivory is higher than the pollination service the interaction becomes unstable showing limit cycles (Figure A.1). The intensity of herbivory depends on the density of larvae and therefore any factors decreasing the maturation rate of larvae will increase herbivory and bring the dynamics to a region of instability. These effects occur in nature: tobacco crops (*Nicotiana attenuata*) can be devastated by tobacco moth larvae (*Manduca sexta*) (Baldwin, 1988). In another theoretical model studying the dynamics of highly specialized mutualistic interaction (i.e. fig-fig wasp) with antagonists (i.e. flower feeding insects) (Wilson et al., 2003), antagonists induce oscillations in the mutualist populations when antagonists visits increase (Bronstein et al., 2003b; Wilson et al., 2003). Therefore, antagonists interactions have a crucial effect on the mutualistic dynamics and they can occur in different life-stages of the pollinator species. A future extension of the model shown in Figure A.1 will be to explore the effects of external antagonists (e.g. herbivore or predator species) and compare it with the antagonistic effects from the pollinator larvae. I expect the conditions to be different because our preliminary analysis already suggests that maturation rate is highly determinant for the stability.

7.2 EVOLUTION AND COEVOLUTION OF MUTUALISTIC SYSTEMS

In mutualistic systems, the complexity of pair-wise interactions has proven to be extremely high because of the interaction and feedback between different life-stages and other species, which can predators, competitors or parasites (Bronstein et al., 2006; Pellmyr, 2003). The consideration of life-history therefore is not only crucial to understand the ecological dynamics, but also the evolutionary dynamics of mutualistic systems, the cost-benefit ratio and their consequences to demography (Holland et al., 2002, 2004b; Ferriere et al., 2007), particularly when more complex cases with multiple interactions are studied (i.e. mutualistic networks).

7.2.1 *Two sides of the same coin: Mutualism-antagonism coevolution*

It has been shown that a combination of mutualistic and antagonistic forces drives the coevolutionary dynamics of plant-animal mutualisms (Jones et al., 2009). The question is what happens when these antagonistic and mutualistic forces are exerted by the same species. The results of the model about mutualism-antagonism of plant-pollinator interactions (Figure A.1) shows this interesting evolutionary dilemma for plants: defend or switch. Plants could either evolve defenses against the herbivory of the pollinator's larvae or find other pollinators to ensure pollination service. For example, tobacco plants (*Nicotiana attenuata*) seem to have evolved both strategies. They have evolved chemical compounds to attract predators (e.g. *Geocoris pallens*) of the pollinator's larvae (*Manduca sexta*) and they have also changed their phenology to attract diurnal pollinators (hummingbirds) (Kessler et al., 2010).

An interesting avenue of research will be to know what conditions promote the evolution of a single (i.e. protection or pollinator switching) or a mixed strategy (protection-switching),

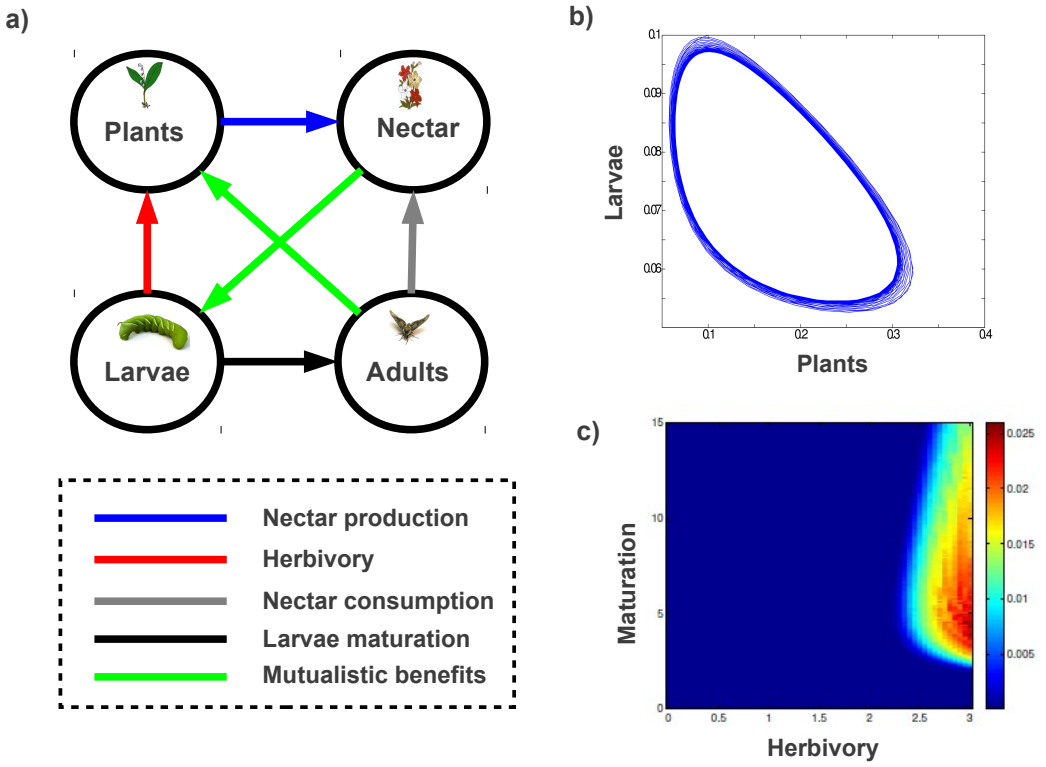

Figure A.1: Model of mutualistic-antagonistic interactions in a plant-herbivore pollinator system. The model is an extension of the model analyzed in Chapter 2 and it describes the complex interaction between insect herbivore pollinators and plants, similar to the interaction between tobacco plants (*Nicotiana attenuata*) and tobacco moths (*Manduca sexta*). Herbivorous larvae (i.e. caterpillars) feed on plant body parts (leaves, stems) to mature and adult pollinators feed on the nectar provided by the plant. a) Model description: the model follows the dynamics of plants and nectar (blue arrow) and the two life-stages of pollinators (larvae and adults). Larvae feed on plants (red arrow) to mature and become adults (black arrow) and adults consume nectar (gray arrow). Mutualistic benefits (green arrows) occur for both plants and pollinators through nectar consumption. b) The model is unstable showing limit cycles when larvae herbivory is higher than the benefits of pollination c) The stability of the model highly depends on larvae maturation rate, as expected from the analysis of Chapter 2. The space parameter shows the amplitudes of population cycles (increasing from blue to red) for different larvae maturation and herbivory rates. Population cycle amplitudes increase when maturation rate is low and herbivory rate is very high. Therefore, under these conditions the mutualism is perilling.

like tobacco plants, using the model explained in Figure A.1. It is known that competition (intra and interspecific) is a critical factor in the ecological and evolutionary dynamics of mutualisms (Ferriere et al., 2007; Jones et al., 2012). Therefore, intraspecific resource

competition between larvae (e.g. leaves) and/or between adults (nectar) will probably determine the eco-evolutionary dynamics of this mutualism. However, it is difficult to predict what conditions allow the stable coevolutionary strategy (coESS); as Jones et al. (2012) state, we still do not know much about the intersection between mutualism and competition.

7.2.2 *Animal behavior and the evolution of plant-animal seed disperser interactions*

Most angiosperm plants depend on the seed dispersal service provided by animals (i.e. endozoochory) (Howe and Smallwood, 1982a). These plants show an extraordinary diversity of complex plant traits, such as fruits; which combine different colors, sizes, aromas and nutritional content (Willson and Whelan, 1990). Empirical research studying the ecology and evolution of plant-animal seed dispersers indicate that animal behavior (Russo et al., 2006; Russo and Augspurger, 2004) and their sensorial biases have largely influenced the evolution of fruits (Schaefer et al., 2007; Flörchinger et al., 2010; Schaefer and Schmidt, 2004). Moreover, Jordano et al. (2007) found that frugivores differ widely in their effects on seed-mediated gene flow. However, there are no theoretical studies exploring the influence of frugivore foraging behavior in the evolution of fruits. This is essential to understand how this amazing diversity of fruit traits has evolved. In Chapter 3 we studied the effect of frugivore behavior on the evolution of animal seed dispersal. The perception and choosiness of the frugivores is crucial for the evolution of fruit traits and therefore for the evolution of this mutualism. Animals with high perception[1] and high choosiness in selecting fruits promote a higher dependence of the plants in the seed dispersal service versus passive seed dispersal [2]. In our model (Chapter 3), the evolution of animal seed dispersal is possible only if frugivores can transport the seeds to better places; i.e. away of the parental tree. Those are places where density-dependence effects are lower and seeds transported by animals have a higher probability to germinate and survive as seedlings. The evolution of animal seed dispersal occurs rapidly when costs of fruit and seed production are not too high. However, as expected, when costs are very high the evolution of fruit traits is difficult to get started and it mainly depends on the initial conditions.

With this chapter I demonstrated the importance of animal foraging behavior for the evolution of animal seed dispersal, as suggested by empirical evidence (Russo et al., 2006; Russo and Augspurger, 2004). The evolution of dispersal syndromes might easily occur in an environment with a diverse array of frugivore animals with different foraging behaviors. Two distinctive animal seed disperser groups with different foraging behaviors are birds and mammals, because of differences in visual and olfactive perceptions and social behavior (Flörchinger et al., 2010; Florchinger et al., 2010; Cazetta et al., 2009). All these potentially contribute to drive the evolution of different fruit traits (i.e. polymorphism) and fruit trait convergence evolution by particular animal seed dispersers (i.e. dispersal syndromes). It remains to study how the evolution of fruit traits will be affected if we consider the dynamics of animal seed dispersers. This could produce eco-evolutionary feedback dynamics between animals and plants (Post and Palkovacs, 2009) and coevolutionary hot spots across space(Thompson, 1999) if spatio-temporal variability is considered.

1 Perception refers to animal sensorial discrimination

2 Seeds not dispersed by animals, falling close to the parental tree

7.2.3 *The evolution of mutualistic networks*

"*Have we not all heard that catechism about genetic drift: it can only be important in populations so small that they are likely to become extinct before playing any sustained evolutionary role* " Gould and Lewontin (1979)

Selective and non-selective forces (i.e. mutation, genetic drift, recombination) contribute to the evolution of traits (Lynch, 2007). The consideration of both is important to understand the evolutionary dynamics and emergence of trait patterns in communities. Recently, various studies have explored the influence of both types of evolutionary forces on the speciation process and eco-evolutionary dynamics of metacommunities (Melián et al., 2010, 2012; de Aguiar et al., 2009). The field of community ecology has moved now to understand the evolutionary processes driving the origin and maintenance of biodiversity (Urban et al., 2008). The multispecific context where species are embedded sets the arena to study coevolution and evolution of species interactions network patterns. Nevertheless, accounting for such enormous complexity is not an easy task. As Levins (1966) says: "Clearly we have to simplify the models in a way that preserves the essential features of the problem". An alternative to studying very complex models based on niche-mechanisms is studying neutral eco-evolutionary models to understand what main processes are behind observed ecological and evolution patterns, in the same way as null models are used to explain ecological patterns.

The mutualistic network literature states that selective forces seem to explain the main ecological and evolutionary patterns (Guimaraes et al., 2011; Thompson and Cunningham, 2002; Thompson, 2009). Empirical evidence shows two main evolutionary patterns in mutualistic webs: convergence and complementarity (Rezende et al., 2007a,b; Bascompte and Jordano, 2007b). Convergence is explained as evidence of common selective pressures by mutualistic partners and complementarity as coevolution between plant and animals species (Bascompte and Jordano, 2007b). There is a strong phylogenetic signal suggesting that network patterns are constrained by past evolutionary history and not exclusively explained by current ecological processes (Vázquez et al., 2009a). Guimaraes et al. (2011) studied a model of coevolution of mutualitic webs and showed that there was evolution of trait convergence and complementarity. However, their model did not consider ecological processes (e.g. dispersal limitation, demography) or specific mechanisms by which species and the interaction network could evolve. Our model (Chapter 6) covers the main factors that influence the evolution of mutualistic webs, in particular neutral eco-evolutionary processes (i.e. ecological and genetic drift, dispersal limitation) and morphological constraints in an individual-based spatially and genetically explicit model. This allows the network of interactions to evolve from a fully genetically homogeneous (i.e. clonal) population. We found that simple mechanisms based on neutral eco-evolutionary processes and morphological constraints for plant reproduction can generate networks with similar topological properties as real plant-animal mutualistic webs. More interestingly, there is evolution of complementarity and convergence in plant (i.e. corolla length) and animal (i.e. proboscis length) traits. Thus, we are able to show that non-selective forces (mutation genetic drift and recombination) and neutral ecological processes (i.e. ecological drift, dispersal limitation)

are also important drivers of mutualistic network evolution. We, however, did not find similar distributions of animal and plant species trait (e.g. corolla and proboscis length) values in real communties (Stang et al., 2009), which tend to be positively-skewed (Stang et al., 2009, 2006). This might be due to the consideration of other important constraints related to plant-animal interaction. For example, there are well known allometries between body size and morphological traits that considerably affect foraging behavior (Kunte, 2007). Kunte (2007) have found that there are important allometric relationships between proboscis length and body size in butterflies (*Lepidpotera*) that could affect their foraging abilities. Species with long proboscis lengths had up to three times longer handling time per flower. Thus, butterflies with relatively long proboscides seem to consume less nectar per unit time from the same flower than butterflies with smaller proboscides (Kunte, 2007). This means that reduced foraging efficiency of pollinators with long proboscides and the competition with other pollinators might limit the evolution of longer proboscis in butterflies. Thus, pollinators with longer proboscides are potentially able to reach the nectar of any flower, but allometric and biomechanical constraints might explain why they have not evolved as generalist pollinators. The importance of allometric relationships in the evolution of plant-pollinator networks still needs to be explored in theoretical studies.

7.3 NEUTRAL OR NICHE PROCESSES?

The scientific community had and still has a debate about the influence of neutral versus niche processes (Ricklefs, 2006; Leibold and McPeek, 2006; Alonso et al., 2006; Rosindell et al., 2012, 2011; Clark, 2012). The debate does not neglect the importance of niche-based processes, but it has moved to understand the feedback on each other (neutral vs. niche) and the strength of these processes in different environments and ecological patterns (Leibold and McPeek, 2006). Although, the Panglossian paradigm[3] (Gould and Lewontin, 1979) seems always present when niche-based arguments are used to explain diversity patterns or community structure, it seems that neutral processes are now discussed and acknowledged as drivers of community structure (Gravel et al., 2006; Holyoak and Loreau, 2006).

In the mutualistic network literature several niche-based processes seem to explain network structure mainly by biological constraints (Bascompte and Jordano, 2007b; Jordano et al., 2003). Behavioral, morphological and phenological traits constraint network interactions making the interaction between some plant and animal individuals more likely than others (Olesen et al., 2010). Phenology in particular seems very important for species interaction (Olesen et al., 2010; Vázquez et al., 2009c) and it is not unique to mutualistic communities (Nakazawa and Doi, 2012). However, as Vázquez et al. (2009a) notes, there is no a single factor that can explain mutualistic network diversity and structure. On the contrary, there are several factors determining the assembly of these communities. Increasing evidence indicates that species abundances, spatial structure, dispersal limitation and stochasticity act simultaneously with niche-based processes shaping the web (Vázquez et al., 2009b,c; Krishna et al., 2008; Canard et al., 2012). It is only now that both neutral and niche processes are being considered (Chapter 6) that we can be more confident about the importance of neutrality and niche-based mechanisms.

3 The Panglossian paradigm refers to the notion that everything has specifically adapted to suit specific purposes

7.3.1 *Spatio-temporal distribution and networks*

In this thesis I found that spatio-temporal distribution is a main determinant of the structure of mutualistic webs (Chapters 4, 5 and 6). The spatio-temporal distribution imposes constraints on the interactions in the network, i.e. species that do not overlap spatially or cannot interact temporally; despite their morphological or body size constraints. These constraints can vary depending on the geographical scale, going from local to regional, and more importantly neutral processes can highly affect spatio-temporal distribution (Hubbell, 2001; Bell, 2000). On the one hand, phenology affects the temporal distribution of species (Olesen et al., 2008). On the other hand, spatial structure and processes (e.g. dispersal limitation) affect local diversity (Hubbell, 2006, 2001). Together, spatio-temporal processes play an essential role in community assembly (Vázquez et al., 2009c,a) and many key network properties seem to emerge from this spatio-temporal variability (Canard et al., 2012; Encinas-Viso et al., 2012).

Phenology has been largely ignored in theoretical studies of community dynamics and ecological networks. In Chapter 4, I showed that considering only this biological constraint several network properties, such as nestedness and connectance, emerge in mutualistic communities. Furthermore, the stability and structure is strongly affected by the distribution of species phenologies and the season length. Season length determines the window of species interactions and it is crucial for the stability and diversity of the community. Climatic drivers, which can dramatically change the distribution of species phenologies can generate detrimental effects on the structure, diversity and stability of the network (Memmott et al., 2007; Hegland et al., 2009). Climate change can produce phenological mismatches altering species interactions and producing trophic cascading effects (Stenseth and Mysterud, 2002; Yang and Rudolf, 2010). However, it is still not clear how robust mutualistic networks are to these changes (Hegland et al., 2009). We found that considering intraguild competition and mutualism, communities living in short season length environments are less resilient and therefore more prone to erode network structure and generate secondary extinction events (Encinas-Viso et al., 2012).

All these changes in the distribution of species phenology are closely linked to the spatial structure and the dispersal abilities of the species. It is well known that spatial constraints limiting the interaction between individuals are also extremely important for community structure. Dispersal limitation can greatly determine the interaction network of a community (Canard et al., 2012). This is what we found in our study on plant-arbucuscular mycorrhizal fungi (AMF) networks (Chapter 5). Contrary to plant-animal mutualisms, both plants and AMF are organisms with very low mobility interacting in the soil and hence their interactions tend to be extremely local (Rosendahl, 2008). We find that the structure of plant-AMF networks can be mostly explained by dispersal limitation and random interactions. Therefore, stochasticity seems to play a major role structuring plant-AMF networks (Dumbrell et al., 2010a,b; Lekberg et al., 2012). Previous studies, which did not consider plant-AMF spatially explicit data, concluded that biological constraints were shaping the structure of plant-AMF networks, like in plant-animal mutualisms (Montesinos-Navarro et al., 2012; Chagnon et al., 2012). Our study sheds light on the importance of considering spatial structure to describe network structure. It remains to study whether

spatial effects alone are more important than other constraints, such as abiotic factors or phenology, shaping plant-AMF network structure.

The importance of spatial effects is not unique for plant-AMF networks. Morales and Vázquez (2008b) found in a simulation study that indeed spatial structure affects several plant-animal mutualistic network properties. For example, it strongly decreases connectance and increases interaction strength asymmetry. I conclude that spatial effects and phenology are both major drivers of the assembly of mutualistic webs. Spatio-temporal distribution is undoubtedly a main determinant of the main network topological patterns, such as nestedness and connectance. Dispersal limitation, immigration and species phenology shape local patterns of mutualistic networks (see Figure A.2), where there is a turn-over of species during the season and across years (Dupont et al., 2009; Olesen et al., 2008). New species entering the network tend to interact with already well-connected species (Dupont et al., 2009). The compelling evidence from my thesis and other studies (Vázquez et al., 2009c; Canard et al., 2012; Dupont et al., 2009) suggest that spatio-temporal variability is the most important driver of mutualistic networks, and probably ecological networks in general. I contend mutualistic network research should start studying spatial effects and phenology together, and not separately.

7.4 THE INTEGRATION OF EMPIRICAL AND THEORETICAL RESEARCH

In the growing field of evolutionary community ecology there is an increasing interest to test models with empirical data (Bolnick et al., 2011; Melián et al., 2012, 2010; de Aguiar et al., 2009). Climate change and other related anthropogenic effects have directed our attention to combine both, empirical and theoretical research. In plant-animal mutualistic communities it is clear that the ecological services provided by animals have not only an important effect on ecosystem functioning and the maintenance of biodiversity (Bascompte et al., 2006; Bastolla et al., 2009), but it also has a strong economical importance for agriculture (Kremen et al., 2002). Recently, the rapid global decline of pollinators set the alarm warning for the devastating effects on the world economy (Biesmeijer et al., 2006; Potts et al., 2010). Fortunately, progress in theoretical and empirical ecological studies are enabling the study of coupled social-ecological systems (Satake and Iwasa, 2009) and the integration of different ecological networks from an ecosystem perspective (Fontaine et al., 2011; Loreau, 2010; Olff et al., 2009). This integration looks for a better predictability that will help to manage and conserve our biosphere.

In the mutualistic network literature, there are not many theoretical models dealing with spatio-temporal variability that allow testing model predictions with data. However, this is a main goal in current research on community and ecosystem dynamics, which also aims to connect different levels of ecological and evolutionary processes. The possibilities of the model studied in Chapter 6, in terms of predictions, are enormous. For example, we can test several models with different eco-evolutionary scenarios and biological constraints. The possible scenarios to study are:

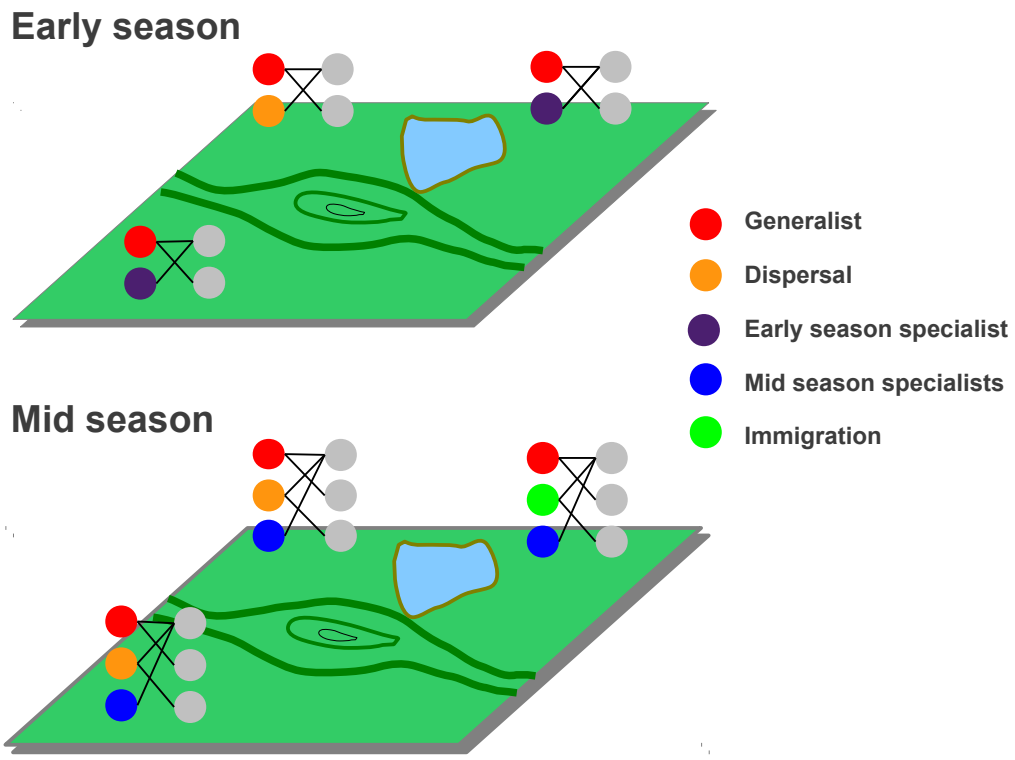

Figure A.2: Spatio-temporal dynamics of mutualistic networks. The spatio-temporal variability of mutualistic webs is moulded by spatial processes (dispersal limitation and immigration) and species phenology, which modifies the local diversity and network structure. In the early season, there are few plant (gray filled circles) and animal (colored filled circles) species and in each site there are generalist species (red filled circle) and early season specialists. Specialist species on each site can vary depending on dispersal limitation. In the middle of the season, species numbers increase due to local dispersal, immigration and the emergence of species by phenology (i.e. species starting to flower). New species (blue and green filled circles) preferentially attach to already well connected species (i.e. generalists), while others disperse to neighboring communities (orange filled circle) or arrive from remote communities (green filled circle).

- NEUTRAL: in this scenario reproduction will only depend on assortative mating and dispersal limitation, i.e. there will not be any biological constraints (or niche-based mechanism) preventing plant-animal interactions. This case assumes that there is no mutualism, each guild will evolve with random interactions between plants and animals. The expected topology will be also highly nested and with low connectance, as shown in previous studies and Chapter 6.

- DOUBLE REPRODUCTION CONSTRAINT: we only considered a morphological constraint for plant's reproduction. However, we could consider a morphological constraint for animal's reproduction as well. Individuals who do not match the corolla length of plant

individuals will not be able to reproduce and survive. This sets a double-constraint for both guilds becoming highly dependent on each other. I expect the possibility of plant-animal coevolution under this scenario, but less diversity than in the studied scenario of Chapter 6. In terms of topological properties, I expect the evolution of modules or compartments across space. This high-compartmentalization is what is observed in real mutualistic webs probably due to high evolutionary convergence and complementarity. The double-constraint will impose selective pressures on both mutualistic guilds generating high trait complementarity and convergence.

Future extensions of the model could also consider pleiotropic and epistatic effects, different assortative mating mechanisms (e.g. self-incompatibility mechanisms), ontogeny (using gene-regulatory networks) or different biological constraints (e.g. phenology, body size). Finally, we could test three eco-evolutionary scenarios (including the scenario of Chapter 6) using Approximate Bayesian Computation (ABC) methods (Beaumont, 2010) and high-resolution empirical data, such as intraspecific trait variation, species abundance distribution and individual-based network topology. For example, Melián et al. (2010) studied different eco-evolutionary models to evaluate adaptive radiations and biodiversity patterns from highly diverse taxonomic groups using ABC. They showed that negative frequency-dependent selection can better explain biodiversity patterns than purely neutral eco-evolutionary dynamics. This (Chapter 6) and other studies (de Aguiar et al., 2009; Rosindell et al., 2010) have opened the door to integrate empirical and theoretical research in evolutionary community ecology. Furthermore, using this model we can also predict phylogenetic relatedness based on genetic differences between species to explore cases of trait convergence and complementarity from real plant-animal mutualistic communities.

7.5 CONCLUDING REMARKS

Mutualistic interactions are very diverse and can be very complex; therefore is difficult to draw general conclusions and predictions by just looking at particular systems, such as plant-pollinator interactions. The life-history of the different mutualistic systems has proven to be highly important to understand their dynamics. Therefore, life-history needs to be accounted for in theoretical models to study in detail simple mutualistic systems (e.g. few species, two or three species interactions), before studying more complex ecological scenarios (e.g. network models). The combination of models with few species and a detailed life-history and network models with simple life-history details is, in my opinion, the key that can provide important insights and set the foundations for a general theory of mutualistic interactions, like has been done in trophic interactions. More importantly, such theory could shed some light on the role of mutualism in community and ecosystem dynamics, for which we still do not know enough. Furthermore, future progress in the mutualistic network area needs the consideration of other ecological interactions and spatio-temporal variability to understand observed network topological properties and improve our predictability.

"You don't need to predict the future. Just choose a future – a good future, a useful future – and make the kind of prediction that will alter human emotions and reactions in such a

way that the future you predicted will be brought about. Better to make a good future than predict a bad one." Isaac Assimov, (Prelude to Foundation)

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